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The Evaluation of Antipyretics Against Pyrogen-Induced Fever*

By MARSHALL R. WARREN and HAROLD W. WERNER†

The subcutaneous injection in rabbits of purified pyrogen prepared from *Proteus vulgaris* produces a fever which lasts several hours. The elevated temperature can be reduced to normal or below by aminopyrine, acetanilid, or acetylsalicylic acid. The rise in temperature can be arrested with acetophenetidin, but this antipyretic does not bring the temperature back to normal. The results may be important in connection with treatment of pyrogen-induced fevers, and they form the basis of a method for evaluating antipyretic activity.

A WIDE VARIETY of substances including peptone, cocaine, tetrahydrobetanaphthylamine, dinitrophenol, thyroxine, vaccines, and bacterial suspensions are employed to produce febrile reactions in animals. Some of the bacterial preparations undoubtedly contain pyrogens, and it is likely that unknown types and amounts of pyrogens are frequently present in extemporaneously prepared solutions employed in the evaluation of antipyretic agents.

It appeared, therefore, that a standardized purified pyrogen prepared from one organism should offer a more satisfactory means of producing fever, and a study was made of the effects of a purified pyrogen following various modes of administration in rats and in rabbits.

Preliminary tests indicated rats were relatively resistant to thermogenic effects of the material employed following intravenous, intraperitoneal, and subcutaneous injections. Rabbits were, as has been demonstrated by others, sensitive to the effects of intravenous injections, but the fever produced did not have sufficient duration for antipyretic studies. It was found that the fever following subcutaneous injections in this species was of satisfactory duration for the purpose of evaluating antipyretic drugs; and the antipyretic action of acetanilid, acetophenetidin, acetylsalicylic acid, and aminopyrine

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† Appreciation is extended to G. C. Bailey and D. G. Marsh of the research section for preparation of purified pyrogen solutions and to C. W. Roberts for technical assistance.

has been determined in rabbits made febrile by the subcutaneous injection of purified pyrogen.

EXPERIMENTAL

Method. The pyrogen was prepared from *Proteus vulgaris* according to the method of Robinson and Flusser (1). The dried material was dissolved in sterile pyrogen-free distilled water in a concentration of 0.015 mg./cc., and chlorobutanol was added in a concentration of 0.5% as a preservative. The solution was sealed in hard glass ampuls and stored at room temperature. Preparations stored under these conditions were found to be highly active when examined after a storage period of eight months. Standardization tests conducted on solutions at that time showed that the threshold pyrogenic dose (dose which caused a rise of 1° F. in rectal temperature of rabbits) was 0.1 cc./Kg. following subcutaneous administration.

Male and female white rabbits weighing between 1.5 and 3.0 Kg. were employed in standardization experiments as well as in evaluations of antipyretic activity. They were housed at approximately 79° F. and were fed on commercial rabbit pellets. Food was withdrawn on the test day two hours before control rectal temperatures were taken. Animals which showed a control temperature greater than 103.5° F. were discarded. Twenty times the threshold dose (2 cc./Kg.) of the pyrogen solution was injected subcutaneously in the mid-dorsal region, and rectal temperatures were taken at hourly intervals for six hours after injection.

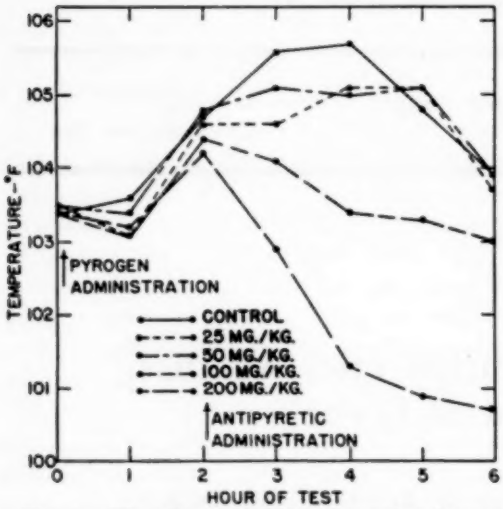

Fig. 1.—The effect in rabbits of varying doses of aminopyrine on fever induced by the subcutaneous injection of twenty times the threshold dose of purified pyrogen.

Six animals were employed as a control group each day tests were conducted, and six animals were used for the evaluation of each selected dose level of the antipyretics. The antipyretic drugs were administered, immediately after the second hourly temperature reading, through a stomach tube as aqueous solutions or suspensions depending on solubility.

RESULTS

Average rectal temperature readings for six pyrogen control animals and for groups of six animals which received various doses of aminopyrine are shown in Fig. 1. Standard errors, calculated for the various hourly readings, range from 0.16 to 0.38 (average 0.25) for the six control animals and from 0.13 to 0.58 (average 0.34) for the various groups of six animals which received aminopyrine. Doses of 25 and 50 mg./Kg. of aminopyrine had only slight effect. Doses of 100 mg./Kg. prevented the expected increase in rectal temperature and gradually decreased body temperature to normal over a period of four hours. The 200 mg./Kg. dose induced a precipitous decline which resulted in subnormal temperatures.

Dosage response curves for acetanilid and acetylsalicylic acid are similar in important respects to those for aminopyrine and standard errors fall within the same range. These two drugs, however, have somewhat lower milligram potencies. Acetanilid prevented further increase in rectal temperature at a level of 100 mg./Kg., but 200 mg./Kg. was required to lower the febrile temperature to normal within the experimental period.

The smallest amount of acetylsalicylic acid which arrested the febrile temperature also reduced it to

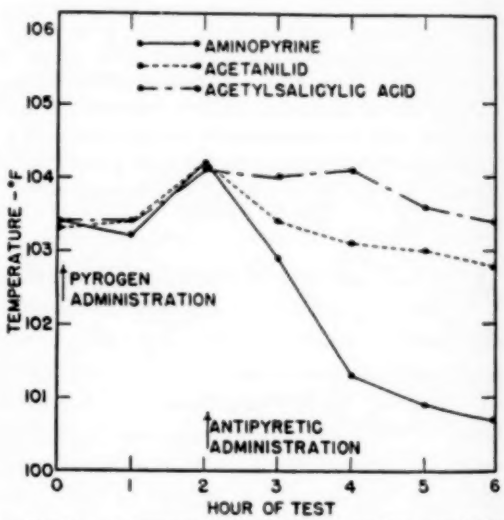

Fig. 2.—Comparative antipyretic action in rabbits of equal doses (200 mg./Kg.) of aminopyrine, acetanilid, and acetylsalicylic acid against fever induced by the subcutaneous injection of twenty times the threshold dose of purified pyrogen.

normal. This dose was 200 mg./Kg. On the basis of minimal amounts required to arrest temperature rises and reduce febrile temperature to normal, the order of decreasing milligram potency for the three most active compounds is: aminopyrine, acetanilid, acetylsalicylic acid.

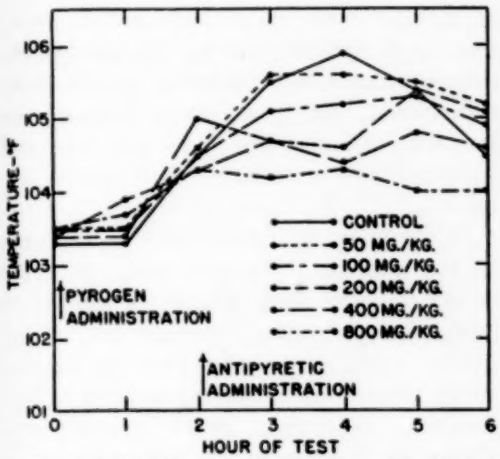

Fig. 3.—The effect in rabbits of varying doses of acetophenetidin on fever induced by the subcutaneous injection of twenty times the threshold dose of purified pyrogen.

This same order of potency is indicated by curves in Fig. 2 which illustrate effects of 200 mg./Kg. doses of these agents. Standard errors, for the various hourly readings, range from 0.13 to 0.32 (average 0.23) for the six animals which received aminopyrine, from 0.20 to 0.31 (average 0.28) for the group which received acetanilid, and from 0.13 to 0.27 (average 0.21) for the group which received acetylsalicylic acid. The 200 mg./Kg. dose of aminopyrine reduced febrile temperature, on the average, to approximately 3° F. below normal. A similar dose of acetanilid caused approximately a 1° reduction below normal, and 200 mg./Kg. of acetylsalicylic acid brought the rectal temperature down to the normal range.

Data in Fig. 3 indicate acetophenetidin was comparatively ineffective against febrile temperatures caused by the pyrogenic substance employed. Doses up to and including a toxic one of 800 mg./Kg. failed to restore rectal temperatures to normal.

The 800 mg./Kg. doses of all four compounds produced toxic effects in some animals. Because of the low incidence of these and the comparatively small number of animals which received the large doses, observed effects do not afford a measure of comparative toxicity. They are recorded, however, because they may be of general interest. Acetanilid, in doses of 800 mg./Kg., produced depression in all animals. A similar dose of aminopyrine caused convulsions and a 50% mortality. Death also occurred in one of six rabbits which received the high dose of acetophenetidin, and in two of six which received acetylsalicylic acid. Abnormally high temperatures, ranging from 107.5° to 109.4° F., developed in one rabbit administered 800 mg./Kg. of acetophenetidin and in two rabbits administered a similar dose of acetylsalicylic acid.

SUMMARY

The antipyretic activity of four well-known antipyretics has been evaluated against fever induced in rabbits by the subcutaneous administration of a purified pyrogen prepared from *Proteus vulgaris*.

Aminopyrine, acetanilid, and acetylsalicylic acid in proper doses arrested temperature rises; and they caused febrile temperatures to return to normal or subnormal levels. Acetophenetidin arrested the temperature increase, but it did not reduce febrile temperatures to normal.

Excessive doses of acetylsalicylic acid and acetophenetidin caused a loss of temperature control in some pyrogen-treated rabbits resulting in higher rectal temperatures than those observed in pyrogen controls. Excessive doses of acetanilide produced depression, and similar amounts of aminopyrine were convulsant.

All compounds, excepting acetanilid, caused some mortality when administered to febrile rabbits in a dose of 800 mg./Kg. Data were not sufficient, however, to establish comparative acute toxicity of the compounds evaluated for antipyretic activity.

REFERENCE

(1) Robinson, E. S., and Flusser, B. A., *J. Biol. Chem.*, 153, 529(1944).

Digitalis. IV. The Collaborative Study of the Assay of Digitalis and Its Preparations by the Chemical and Cat Methods*,†

By FREDERICK K. BELL and JOHN C. KRANTZ, JR.

SOME MONTHS ago a chemical method for the assay of digitalis preparations was published (1). This method, based upon the colorimetric determination of the active glycosides by means of the Baljet color reaction, was found to be very successful in the hands of a limited group of collaborators.

With regard to other colorimetric methods previously proposed, the authors claimed several distinct advantages for their procedure. The use of a photoelectric colorimeter eliminates a number of troublesome factors which frequently occur when the ordinary visual type of instrument was employed. By preparing the color standard from U. S. P. Reference Standard Digitalis Powder several obvious advantages were obtained. From an extensive study of the influence of temperature and the time on the development of the color reaction definite specifications for the procedure were established. Confirming earlier work, it was found that the intensity of color reaction of the three important glycosides of *Digitalis purpurea* was, in general, proportional to the cardiotonic activity. In comparison with the available bioassay methods, this colorimetric procedure offered definite advantages with regard to the time, cost, and simplicity of the assay.

It is therefore not surprising that the United States Pharmacopœia found that the method was worthy of consideration and that an extensive collaborative study was undertaken under the direction of Dr. E. Fullerton Cook, Chairman of the Committee of Revision. Ten coded samples of digitalis preparations which included new and old powders, new and old tinctures, tablets and capsules, were supplied each collaborator. As of the date of this communication Chairman Cook had received reports from thirty collaborators which included government, educational, and commercial laboratories. It is the purpose of this paper to give a brief summary and analysis of the results obtained.

Of the thirty collaborators, two reported on the bioassay only, and twelve reported on the chemical method only. A total of 18 laboratories reported bioassays and 28 reported chemical assays of which 25 assayed all 10 preparations, one reported on 7 preparations, and two on 9 preparations. Therefore there were 275 preparations assayed by the colorimetric method. There were 42 preparations bioassayed allowing for duplications. A grand total of 281 preparations were assayed. Strictly considered, these totals are subject to a correction because two samples were tinctures and therefore in the form required for the assays. Presumably all laboratories were assaying the same preparations in the case of each of these two samples.

From the reports, apparently very little difficulty was encountered with the chemical assay procedure and all of the better known types and makes of photoelectric colorimeters were employed. The ten coded samples distributed by Chairman Cook to the collaborators included the following: 4 specimens of powdered leaf; 1 specimen in the form of capsules; 3 specimens in the form of 1.5-grain tablets and 2 tinctures.

A summarized tabulation of the results is shown in Table I in which the number of assays and the high, low, and average values obtained for each sample by each of the two assay methods are given. All assay values have been rounded off to the nearest unit. In interpreting the results several points should be borne in mind. The gross disparity between the number of assays carried out on each sample by the two methods was

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† The expense of this study was defrayed in part by a grant from the Board of Trustees of the United States Pharmacopœial Convention.

unavoidable for reasons of practicality and therefore the range of variability is not strictly comparable. Presumably the collaborators who undertook the bioassays were skilled in that method and also in the pharmaceutical procedure involved in preparing the various tinctures. On the other hand, most of the laboratories which reported on the chemical method probably were using that procedure for the first time.

The results obtained for Sample 3 by the two methods are in satisfactory agreement. The chemical method yields a value 10 points higher than the biological assay. For Sample 4 the chemical method gives a value 50 points higher than the biological assay.

While the final averages for the assay of the capsules of Sample 5 by the two methods are in good agreement, a much higher variation is shown in the chemical values. This is

TABLE I

	Chemical Method				Bioassay Method			
		Assay Value				Assay Value		
Sample No.	No. of Assays	High	Mean	Low	No. of Assays	High	Mean	Low
1	28	156	90	65	5	104	92	74
2	27	76	69	59	3	76	71	63
3	28	129	114	100	5	109	94	87
4	27	215	175	130	4	148	125	110
5	25	199	85	37	5	100	90	82
6	27	185	134	105	6	130	120	91
7	28	179	110	72	2	105	96	87
8	28	199	103	46	5	101	94	87
9	28	117	101	87	3	100	92	78
10	28	150	117	102	5	112	86	72

The average values obtained for the powdered leaf samples Nos. 1 and 2 are practically in perfect agreement by the two methods. The difference of two percentage points in each case is less than the limit of reproducibility of either method. With Sample 1, the much wider range of variation in the chemical assay values is accounted for by three values of 156, 65, and 65 which, incidentally, have an average of 95. The remaining 25 assays ranged in value between 75 and 101.

Since Sample 2 was composed of 75% of Sample 1 and 25% exhausted marc these assay values give an interesting check on the validity of both methods. The ratio of the potencies of Sample 1 to Sample 2 gives an ideal value of 1.33. Only one laboratory reported bioassays for both of these samples and their ratio was 1.21. The average value of the ratio for all bioassays of Samples 1 and 2 was 1.30. The chemical assay of both of these samples was reported by 27 collaborators and the three extreme values for the assay of Sample 1 previously noted are included therein. In these three cases the potency ratio is obviously completely out of the range of the average. Nevertheless the grand average of this ratio by the chemical method is 1.30.

accounted for largely by 4 values, *viz.*, 37, 42, 48, and 199. These values have an average of 81 and it is interesting to note that the application of a factor of 2 would bring each value in line. The remaining 21 values lie between 60 and 106.

The assay values for the 1.5-grain tablets of Samples 6, 7, and 8 by the two methods are in fair to good agreement. Unusual variations in the chemical assay values for Sample 8 are largely due to values of 199, 46, 48, 48, which give an average of 85. Again it is to be observed that the use of a factor of 2 would bring each of these values in line. The remaining 24 values ranged between 95 and 135.

In the chemical assay of the capsules and tablets represented by Samples 5, 6, 7, and 8, a particularly important factor is involved. Each of these samples presumably contains 0.1 Gm. of powdered leaf per capsule or tablet. From the weight of each preparation we have calculated the approximate composition as follows:

	Powdered Leaf, %	Excipient, %
Sample 5, Capsules	45	55
Sample 6, Tablets	83	17
Sample 7, Tablets	62	38
Sample 8, Tablets	40	60

It is well known that the Baljet color reaction is not specific for the digitalis glycosides and their genins. For example, certain sugars react readily to give the characteristic color. We do not know what excipients were used in these four preparations but lactose is among the most favored substances for this purpose.

From studies which we have recently completed it was found that even 0.1 per cent solutions of lactose and dextrose show an appreciable color reaction with the Baljet reagent and that the reaction is especially sensitive to temperature changes. On the other hand, sucrose and mannitol were found to be inactive. In preparing tinctures from preparations containing appreciable amounts of excipients which are active toward the Baljet reagent it seems certain that significant amounts of such excipients could be extracted by the 70 per cent alcohol used as a menstruum, and therefore carried on through to the final test solution. Until more complete information is available the chemical method should be used with caution in the assay of digitalis preparations containing excipients.

In the matter of excipients another point worthy of consideration in connection with the collaborative study should be mentioned. It seems highly probable, from the detailed reports, that some collaborators prepared their tinctures from the capsules and tablets on the basis of their actual weight rather than on the expected weight of powdered leaf contained in each preparation.

The average assay values obtained by the two methods for the tincture represented by Sample 9 are in good agreement. On the other hand, those obtained for the tincture of Sample 10 show a difference of 31 units. The highest value reported by the chemical method was 150 which exerts no significant effect on the final average. The next highest value reported for this assay was 129.

There were 34 preparations assayed by both methods in the first months of the study. These data were analyzed statistically. Of these results the mean for the chemical method was: 113 per cent $\sigma = 40$ and C. V. = 35.4. By the cat method the mean was: 95 per cent $\sigma = 26$ and C. V. = 26.8. The correlation coefficient of the 34 pairs was:

$$r = 0.71 \pm 0.12 \text{ or P. E.} = 0.08$$
$$0.71/0.08 = 8.9$$

r is 9 times greater than P. E. which suggests significant correlation and that essentially each method is measuring the same factor. Or in other words, the correlation between the two methods in this series is high, indicating with a certainty of approximately 30 per cent greater than sheer chance that these two methods are measuring the same activity.

Additional experimental data concerning the relation between the two methods of assay are still very limited. Vos and Welsh (2) have reported results on 16 tinctures and 8 varieties of tablets and capsules. In 21 of these samples they report fair agreement between the two assay methods, with the colorimetric method giving higher values in general. Gross discrepencies were observed with three samples all of which were tinctures and which gave values over 50 per cent above the bioassay value.

In a private communication from Dr. M. G. Allmark of the Laboratory of Hygiene in the Department of National Health and Welfare at Ottawa, Canada, we have obtained the following data. Ninety-six per cent of the chemical assays made on 46 digitalis preparations consisting of powdered leaf, capsules, and tablets gave values which agreed with those of the official cat method within 25 per cent or less. Of the chemical assays on 19 tinctures, 78.9 per cent fell in the same category.

As has already been mentioned, difficulty might be expected in the chemical assay of capsules and tablets which contain excipients that give the characteristic color reaction. However, the foregoing two reports indicate that the chemical method is less reliable in the assay of tinctures than in the dried leaf preparations. This presents an interesting problem. Since the assayist must prepare tinctures of the dried leaf preparations for use in the chemical assay, why do these tinctures appear to give more reliable results than those obtained from commercial tinctures? There appears to be no consistent

relationship between the age of the tincture and its chemical assay value. Besides, the Pharmacopœia prohibits the addition of any foreign substance to the official tincture. Further studies will be required to determine the source of the difficulty, but at this point it would appear that the purity of the alcohol used for the menstruum should receive particular attention.

Concerning the higher results generally obtained by the chemical method as compared with those by bioassay, we believe the most important factor is the presence of diginin. This glycoside, isolated by Karrer (3), has practically no cardiotonic activity but does give a positive reaction with the Baljet reagent. Perhaps other substances playing a similar role are still to be isolated.

REFERENCES

(1) Bell, F. K., and Krantz, J. C., Jr., *J. Pharmacol.*, **83**, 213(1945).
(2) Vos, B. J., Jr., and Welsh, L. H., *ibid.*, **85**, 346(1945).
(3) Karrer, W., "Festschrift," Emil C. Barell, 1936, p. 239.

Pentobarbital Sodium Anesthesia in the Syrian Hamster*

By FRANK J. ORLAND and PHYLLIS M. ORLAND

The Syrian hamster (*Cricetus auratus*) was studied to determine an effective dosage of pentobarbital sodium in relation to the length and depth of general anesthesia produced. In general, the hamster has an apparently greater tolerance to pentobarbital sodium both in anesthetic and lethal doses than the rat, guinea pig, or rabbit. No sex variation in reaction to the drug was observed in the hamsters studied.

THE SYRIAN HAMSTER, *Cricetus auratus*, a relatively new laboratory animal already being used in a great variety of experimental investigations, was studied to determine an effective dosage of pentobarbital sodium in relation to the length and depth of general anesthesia produced.[1]

In a preliminary survey, the actions of several parenterally administered barbiturates upon hamsters were studied. However, it was soon found that pentobarbital sodium given by intraperitoneal injection was more effective than sodium isoamyl-ethyl barbiturate (Amytal) and phenobarbital sodium when given by the same route as well as pentobarbital sodium when given subcutaneously.

* Received April 24, 1946, from the Walter G. Zoller Memorial Dental Clinic and the Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.

EXPERIMENTAL

A solution of pentobarbital sodium[2] (Nembutal) was prepared so that 1 cc. was equivalent to 10 mg. of the drug. Calibrated, 1-cc. syringes with 21- to 23-gauge needles were used for the intraperitoneal injections. The animals were weighed and the drug dosage calculated on the basis of 100 Gm. body wt., since that was near the actual weight of the average adult hamster. Weights of the animals studied varied from 30 to 114 Gm. and ages from two to six months. Forty-two males and 18 females, all normal, were given injections of the drug; a few pregnant females were likewise studied.

In the technique of making the intraperitoneal injections, the animal was held by the loose dorsal skin with the left hand of the operator, so the abdominal skin was tense and accessible. The area around the proposed site of injection was thoroughly wetted with 70% ethyl alcohol. Next, the needle, firmly attached to the syringe containing the proper amount of drug, was introduced $^1/_2$ to 1 cm. above Poupart's ligament, in the lateral abdominal area, and directed anteriorly and internally until a sudden release of tension revealed intraperitoneal placement. The drug was then injected and the needle withdrawn. No evidence of peritonitis or subcutaneous infection was noted, although the same needle was used for injecting several animals.

[1] The need in this laboratory for various degrees of general anesthesia which could be easily and safely administered to the hamster for procedures involved in the study of experimental dental caries prompted this investigation.

[2] Freshly prepared solutions of the drugs were used in all instances except for a preparation of Veterinary Nembutal, alcohol 10% (Abbott), which per mg. gave the same results as the freshly prepared pentobarbital sodium.

RESULTS

From the accompanying diagram (Fig. 1) it is evident that very satisfactory and predictable anesthesia was produced by intraperitoneally administered pentobarbital sodium. Up to 1 mg. per 100 Gm. body wt. no effect was noted; with 1 to 3 mg. per 100 Gm. an increasing amount of excitement, both in duration and intensity, was observed; with 3- to 5-mg. doses, short third-stage anesthesia was produced lasting from five to twenty-five minutes. As the doses were increased, the duration also increased, so that with 8 mg. an average of sixty minutes of complete surgical anesthesia was attained. Doses as high as 15 mg. per 100 Gm. body wt. were given with complete recovery. One animal recovered even after thirteen hours of deep anesthesia produced by 14 mg. per 100 Gm. Another animal, however, died with 10 mg. per 100 Gm. body wt., and two died with doses of 13 and 14 mg.

From the above data it is apparent that one can reasonably well predict the depth and duration of anesthesia with intraperitoneal pentobarbital sodium, and that a very safe range between 5 and 9 mg. per 100 Gm. body wt. will usually assure satisfactory surgical anesthesia from 15 to 75 minutes.

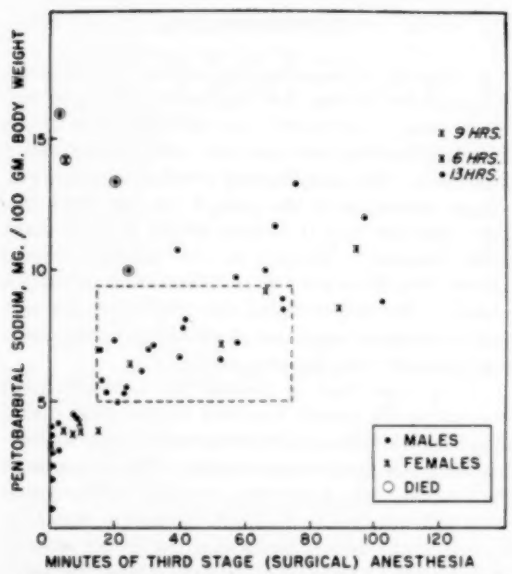

Fig. 1.—Illustrating the duration of third-stage anesthesia in the Syrian hamster with increasing doses of pentobarbital sodium administered intraperitoneally. (Three animals remained anesthetized for thirteen, six, and nine hours and completely recovered.) The above distribution indicates a safe and predictable range using between 5 and 9 mg. which assures surgical anesthesia from fifteen to seventy-five minutes.

DISCUSSION

Since no work regarding barbiturates in hamsters has been reported, there may be some value in comparing the reactions observed with those found in other rodents. According to Griffith (1) 4–5 mg. pentobarbital sodium per 100 Gm. body wt. is given the rat for deep surgical anesthesia lasting forty-five minutes. Kinsey (2, 3, 4) found that 31.3 mg. per Kg. body wt. given intraperitoneally produces 79 minutes and 231 minutes of anesthesia in male and female rats, respectively; 15.6 mg. per Kg. of guinea pigs anesthetizes them for 88 minutes, while 40–44 mg. per Kg. in rabbits produces an average of 204 minutes of anesthesia. An anesthetic dose of 30–40 mg. and 40 mg. per Kg. of rabbit is reported by Mulinos (5) and Fitch and Tatum (6), respectively. On the basis of these comparative reactions to pentobarbital sodium, it seems that the hamster has greater tolerance for the drug than many other laboratory animals.

The M. L. D. per 100 Gm. body wt. of the hamsters studied was approximately 13.5 mg. of intraperitoneally administered pentobarbital sodium. This is a somewhat larger dose than that for rats, 120 mg. per Kg. body wt., as determined by Swanson (7) and Barlow (8), and 75 mg. per Kg. according to Fitch and Tatum (6). The M. L. D. for guinea pigs reported by Carmichael and Posey (9) is 45–60 mg. intraperitoneally and that for rabbits is 65 mg. as determined by Fitch and Tatum. When lethal doses were given to hamsters, the time elapsing until death was inversely proportional to the amount of the lethal dose; for example, an animal given 13.4 mg. per 100 Gm. body wt. died in twenty-seven minutes while one given 16.1 mg. died in seven minutes. Irregular respiration as low as 20 per minute, and a heart rate of 80 per minute were still compatible with complete recovery; whereas normal respiration for actively moving hamsters was approximately 200 per minute and for sleeping animals approximately 70, while the normal heart rate as measured by electrocardiography averaged 400 per minute.

Too few hamsters had repeated injections at frequent intervals to note any increase in tolerance to pentobarbital sodium although Moir (10) reported that rats repeatedly injected with the same drug developed a tolerance, while Carmichael and Posey (9) reported a similar tolerance developing in

TABLE I.—STAGES OF ANESTHESIA IN THE SYRIAN HAMSTER INDUCED BY PENTOBARBITAL SODIUM GIVEN INTRAPERITONEALLY

Stages	Animal Reaction	Approximate Rate per Minute		Average Time, Min.		
		Resp.	Heart	Onset After Injection	Duration[a] Descending	Ascending
I, from injection to excitation	Normal	200	400	....	1– 1.5	.....
II, excitation	Excitation and gradual loss of coordination	rapid and irregular	430	1–1.5	0.5–3	20–60
III, surgical						
Plane 1	Vibrissae active, intercostal resp., corneal reflex, mass movement induced by noxious stimuli	60–80	...	2– 4	4–25	5–10
Plane 2	Vibrissae inactive, abdominal resp., corneal reflex, slight reaction to noxious stimuli	40–60	360	4– 8	4–60+	10–60+
Plane 3	Sluggish corneal reflex, shallow abdominal resp., slight cyanosis, no reaction to stimuli	20– 40	...	6–10	10–60+	10–60+
Plane 4	All reflexes abolished, deep, spasmodic resp., marked cyanosis	4– 16	120	7–15	2.5	.....
IV, respiratory arrest	Cessation of resp., later—cardiac arrest, death	0	60, irreg.	4–30	0.5 with recovery; 2–3 to death	.....

[a] Duration of stages and planes of anesthesia were variable, depending primarily on dosage. The larger the dose, the more sudden was the descent through the higher planes and the longer was the duration in the lower planes unless the animal died. The length of the first and second stages in descent remained within a rather narrow range regardless of dose. However, in ascent the excitatory period was longer for larger doses and also relatively long for small doses which did not anesthetize the animal beyond the excitatory stage.

guinea pigs. Pregnancy in the hamster seemed to slightly increase tolerance; this was probably due to the increased metabolic rate of animals in such a physiological state.

No sex variation in reaction to the drug was observed in the hamsters studied. Observations on rats by Carmichael (11) and on rabbits and rats by Fitch and Tatum (6) regarding median lethal doses also showed no sex difference, while Kinsey (3, 4) noted no sex difference when anesthetic doses were given to guinea pigs and rabbits. On the other hand, Moir (10) and Holck and Kanan (12) indicate female rats to be more sensitive than male rats to the drug as judged by sleeping time and as judged by both sleeping time and M. L. D., respectively; furthermore, Kinsey (2) pointed out that female rats sleep twice as long as male rats with either single or repeated injections of the drug.

Differences in weights of hamsters did not seem to alter the duration or depth of anesthesia, an observation similar to that of Carmichael (11) on rats. A comprehensive review of barbiturates was written by Tatum (13) in 1939, wherein he describes the reaction to this group of drugs of many available animals but not of the hamster.

Table I shows the duration and objective signs of the various stages and planes of anesthesia in the hamster on the basis of observation on animals anesthetized with pentobarbital sodium given intraperitoneally.

SUMMARY

1. The use of pentobarbital sodium when administered to the Syrian hamster by intraperitoneal injection provided a wide range of safe, predictable, third-stage (surgical) anesthesia.

2. The reaction of the hamster to pentobarbital sodium as compared to those of other rodents was discussed.

REFERENCES

(1) Griffith, J. Q., and Farris, E. J., "The Rat in Laboratory Investigation," J. B. Lippincott Co., Philadelphia, 1942, p. 20.
(2) Kinsey, E. V., THIS JOURNAL, **29**, 387 (1940).
(3) *Ibid.*, p. 342.
(4) *Ibid.*, p. 292.
(5) Mulinos, M. G., *Arch. intern. pharmacodynamie*, **47**, 111 (1934).
(6) Fitch, R. H., and Tatum, A. L., *J. Pharmacol.*, **44**, 325 (1932).
(7) Swanson, E. E., and Shonle, H. A., *J. Lab. Clin. Med.*, **16**, 1056 (1931).
(8) Barlow, O. W., Duncan, J. T., and Gledhill, J. D., *J. Pharmacol.*, **41**, 367 (1931).
(9) Carmichael, E. B., and Posey, L. C., *ibid.*, (*Proc.*) **57**, 116 (1936).
(10) Moir, W. M., *ibid.*, **59**, 68 (1937).
(11) Carmichael, E. B., *ibid.*, **62**, 284 (1938).
(12) Holck, H. G. O., and Kanan, M. A., *Proc. Soc. Expll. Biol. Med.*, **32**, 700 (1935).
(13) Tatum, A. L., *Physiol. Revs.*, **19**, 472 (1939).

Dihydroxypropyl Theophylline: Its Preparation and Pharmacological and Clinical Study*

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The preparation, identification, and properties of dihydroxypropyl theophylline are described. The results of acute and chronic toxicity studies indicating its safety under ordinary conditions of use are reported. Pharmacological tests purporting to show certain possible advantages of dihydroxypropyl theophylline over some other xanthine derivatives used as drugs are reported.

INTRODUCTION

THE clinical use of the xanthine derivatives caffeine, theobromine, and theophylline has been established for some time. However, there has been a considerable difference of opinion among members of the medical and allied professions as to the value of theophylline and its derivatives in clinical medicine.

In response to many inquiries from the members of the medical profession requesting information in regard to the value of theophylline and its derivatives in certain pathological conditions, the Council on Pharmacy and Chemistry of the American Medical Association in 1930 (1) published a report, "Therapeutic Claims for Theobromine and Theophylline Preparations." The permitted claims for these drugs as stated by the Council for Theophylline in this report were:

(*a*) Diuretic action.
(*b*) Myocardial stimulation.
(*c*) Relief of pain in angina and similar lancinating pains.

Early clinical reports appear in the German literature (2) wherein it was found that caffeine, theobromine, and theophylline all have an active vasodilator action on the coronaries, probably muscular in origin. Heathcote (3) reported similar action of vasodilation by these xanthine derivatives. Smith, Miller, and Graber (4) employed the isolated rabbit's heart and concentrations of the drugs estimated to be within the range that may reasonably be expected in the blood. After administration in man, they found little or no effect from caffeine or theobromine but a marked increase in coronary flow was observed when theophylline was used. One of the first to recommend the xanthine diuretics in the treatment of angina pectoris was Askanazy (5). In 1895 he found occasional striking results with patients in the treatment of cardiac asthma with or without angina pectoris. His observations were corroborated by Breuer (6). In the report by Theohari (7), summaries on ten cases of hypertension were reported in which benefit from the xanthines was obtained. Theohari believed that theocin (theophylline) was of value in producing a diminution in original attacks of cardiac failure, and also in causing a fall in blood pressure. In a study of euphylline (theophylline ethylenediamine) by Smith, Graber, and Miller (8) it was found that the drug gave relief in six out of ten cases with angina.

Although the therapeutic value of theophylline has been established, the drug possesses the disadvantage of being very insoluble. In order to increase the clinical usefulness of theophylline, several mixtures have been prepared which increase its solubility. These mixtures include theophylline ethylenediamine, theophylline sodium acetate, and theophylline sodium salicylate (9).

In the case of theophylline ethylenediamine, the high alkalinity of the mixture not only renders the compound incompatible with many other drugs but also might account for the great number of gastric upsets experienced by Boyer (10) and other clinicians. It is also known that the gastric juice, being acidic, precipitates a consider-

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able amount of theophylline from theophylline ethylenediamine and thus destroys the solubility advantage.

The object of this work was to prepare a soluble theophylline derivative which would retain the therapeutic activity of theophylline yet be devoid of the incompatibilities and nauseating effects experienced by the mixtures now available.

Two hydroxy derivatives of theophylline, hydroxyethyl theophylline and theophylline glucoside, were prepared according to methods outlined in part in German patents, numbered (11) and (12), respectively. Although these derivatives were found to be very soluble, they were apparently not subjected to pharmacological investigation to determine their therapeutic activity. Therefore, the above compounds were prepared and studied pharmacologically as a basis for the advisability of preparing similar theophylline derivatives. On the basis of the results obtained in these pharmacological studies, a new derivative of theophylline, dihydroxypropyl theophylline, was prepared and investigated both pharmacologically and clinically.

EXPERIMENTAL

Preparation of Dihydroxypropyl Theophylline.—One hundred and eighty grams of theophylline, dried at 100° for two hours, was dissolved in 500 cc. of boiling water. Forty grams of sodium hydroxide was then carefully added with constant stirring. When solution was complete, 120 Gm. of 3-chloro-1,2-dihydroxypropane was added slowly with constant stirring. The mixture was boiled under normal atmospheric pressure for two hours, during which time the temperature gradually increased to 110°. The liquid was then evaporated under reduced pressure to remove the last trace of water. The heavy, syrupy, clear liquid remaining became cloudy after standing three days and, when stirred, soon formed a solid mass.

The theophylline compound was separated and purified in the following manner:

Three hundred grams of the solid was reduced to a thirty mesh powder and added to one liter of boiling absolute methyl alcohol. The mixture was boiled for five minutes to completely dissolve the soluble portion of the reaction mass, then filtered.

The clear filtrate was again boiled for three minutes, then allowed to stand overnight at room temperature.

Dihydroxypropyl theophylline was thus obtained as a white, odorless, and extremely bitter powder. Its melting point was 155–157°. Analysis for nitrogen gave 21.97%. The calculated nitrogen content is 22.04%.

The sodium chloride formed by the reaction between theophylline sodium and 3-chloro-1,2-dihydroxypropane was isolated and purified. The yield was 95% of the amount required by theory.

One gram of dihydroxypropyl theophylline is soluble in 3 cc. of water, 70 cc. of absolute ethanol, or 35 cc. absolute methanol at 25°. One gram dissolves in 10 cc. of boiling absolute ethanol and in 6 cc. of boiling absolute methanol.

Diacetylpropyl theophylline was prepared from dihydroxypropyl theophylline, acetic anhydride, and glacial acetic acid. The formation of the diacetylpropyl theophylline established the presence of two hydroxy groups in the molecule of dihydroxypropyl theophylline. Analysis for nitrogen content by the Kjeldahl method gave 16.48%. The calculated content is 16.57%.

EXPERIMENTAL PHARMACOLOGY AND TOXICOLOGY

Theophylline toxicity varies with the route of administration in general, and likewise with the physical and chemical properties of derivatives used clinically. Goodman and Gilman (13) state that theophylline and all other xanthines are irritating to the gastric mucosa. In oral therapeutic doses they are likely to cause a gastric discomfort, nausea, and vomiting and often it is impossible to give the desired amount, the doses in many cases being limited to the extent of gastric tolerance. However, Adams (14) has pointed out, in connection with the intravenous administration of aminophylline, a number of deaths which are attributed to the drug itself.

Although theophylline and its derivatives are not free from toxicological manifestations, interest and usage are on the increase. Having outlined the toxicology as well as the accepted uses for known theophylline derivatives, a basis has been established for pharmacological and clinical studies for the drug dihydroxypropyl theophylline.

Toxicity Studies

Oral Administration.—An attempt to establish a toxic oral dose of dihydroxypropyl theophylline was made, and at the same time to compare its relative toxicity with sodium theophylline, hydroxyethyl theophylline, theophylline glucoside, and aminophylline.

One hundred and forty rats were given systematically arranged doses of dihydroxypropyl theophylline. The drug was administered in aqueous solution by means of a stomach tube. During period of observation in the metabolism cages no food or water was given. These data indicated a very low toxicity of dihydroxypropyl theophylline when administered orally. Although the highest dose (400 mg./Kg.) is twenty times the determined optimum clinical therapeutic dose, no deaths resulted.

With dosages within the therapeutic range (20–60 mg./Kg.), the rats were quite normal in their behavior, and as soon as they were returned to the stock cages feeding was usually resumed at once. Doses from 100–400 mg./Kg. produced little or no evidence of central nervous system excitation. Observation of the rats up to a period of thirty days after tests disclosed no atent toxicity symptoms. In most clinical conditions, theophylline compounds are administered in fairly high doses over a period of several days.

To determine whether toxic symptoms develop in rats when given large doses of dihydroxypropyl theophylline daily over a period of time, four rats were given 250 mg./Kg. (12.5 times the therapeutic dose daily) for a period of thirty days. The drug was administered each morning by stomach tube, and the rats then returned to their cage where they were given the regular stock diet of Purina dog biscuits and water. There were no deaths and all rats continued to gain weight. During the period of toxicity tests the rats evidenced little central stimulation. Histological examination of the kidneys at the completition of the test disclosed no tissue damage.

To compare the oral toxicity of dihydroxypropyl theophylline with other theophylline derivatives, the following drugs were selected: Aminophylline, theophylline glucoside, theophylline sodium, and hydroxyethyl theophylline. The dosage used in each case and results obtained are outlined in Table I.

TABLE I.—DIHYDROXYPROPYL THEOPHYLLINE TOXICITY AS COMPARED WITH KNOWN THEOPHYLLINE DERIVATIVES

Drug	Dose, Mg./Kg.	No. of Rats	No. of Deaths at End of 24-Hr. Period
Dihydroxypropyl theophylline	25	2	0
	50	2	0
	100	2	0
	200	2	0
	400	2	0
Theophylline glucoside	25	2	0
	50	2	0
	100	2	0
	200	2	0
	400	2	0
Hydroxyethyl theophylline	25	2	0
	50	2	0
	100	2	0
	200	2	0
	400	2	0
Aminophylline	25	2	0
	50	2	0
	100	2	0
	200	2	2
	400	2	2
Theophylline sodium	25	2	0
	50	2	0
	100	2	0
	200	2	1
	400	2	2

Intravenous Toxicity.—While this work was intended primarily for the development of a theophylline derivative for oral administration, it was deemed important to have available data relative to the toxicity of dihydroxypropyl theophylline when administered intravenously.

Corresponding doses of aminophylline were administered for relative toxicity. The injections were made into the saphenous vein, and the doses were arranged systematically as outlined in Table II.

TABLE II.—TOXICITY TESTS INTRAVENOUS

Dose, Mg./Kg.	No. of Rats	No. of Deaths
Dihydroxypropyl Theophylline		
20	2	0
30	2	0
40	2	0
50	2	0
100	2	0
240	2	0
Aminophylline		
50	2	0
100	2	0
240	2	0

TABLE III.—COMPARISON OF DIURETIC ACTIVITIES OF DIHYDROXYPROPYL THEOPHYLLINE WITH STANDARD UREA AND AMINOPHYLLINE

Drug	No. of Sub-groups Rats Used	Dose, Mg./Kg., in saline, 50 Cc./Kg.	Average Per-centage Secretion in Five hr.	Average Diuretic Action
Saline	4	50	38.5	1.00
Urea	2	250	38.6	1.00
Urea	2	500	41.1	1.06
Urea	2	750	55.1	1.42
Urea	2	1000	70.0	1.78
Dihydroxypropyl theophylline	2	10	38.9	1.01
Dihydroxypropyl theophylline	4	20	70.2	1.82
Dihydroxypropyl theophylline	2	30	61.5	1.59
Dihydroxypropyl theophylline	2	40	56.6	1.46
Aminophylline	2	10	38.5	1.00
Aminophylline	3	20	68.4	1.77
Aminophylline	2	30	62.5	1.59
Aminophylline	2	40	52.3	1.35

Stability Tests in Gastric and Intestinal Juice.—The fact that aminophylline depends upon highly alkaline ethylenediamine for its solubility raised the question as to its stability in the fluids of the gastric intestinal tract. Using accepted artificial gastric and intestinal fluids, 5% concentrations of dihydroxypropyl theophylline and aminophylline were subjected to digestive action which simulated actual conditions encountered in the intact animal. There was no precipitation of theophylline by action of the digestive fluids in the case of dihydroxypropyl theophylline, but 75% of the aminophylline was precipitated in the gastric juice in a period of six hours.

TABLE IV.—EFFECT OF DIHYDROXYPROPYL THEOPHYLLINE ON BLOOD VESSELS OF TURTLE AS COMPARED WITH KNOWN THEOPHYLLINE DERIVATIVES

Drug	Clock Time	Flow per Min. in Drops
Normal	10:15	6
	10:16	7
	10:17	7
	10:18	9
	10:19	8
	10:20	7
Theophylline, 1 cc. (1–1000)	10:30	18
	10:31	27
	10:31	30
	10:32	18
	10:33	13
	10:34	12
	10:35	9
	10:36	9
	10:37	7
Dihydroxypropyl theophylline, 1 cc. (1–1000)	10:40	10
	10:41	22
	10:42	26
	10:43	16
	10:44	14
	10:45	10
	10:46	10
	10:47	10
	10:48	9
	10:49	8
	10:50	7
Theophylline glucoside, 1 cc. (1–1000)	10:53	8
	10:54	18
	10:55	18
	10:56	13
	10:57	9
	10:59	8
	11:00	7
Aminophylline (1–1000) 1 cc.	11:03	8
	11:04	18
	11:05	25
	11:06	21
	11:07	15
	11:08	10
	11:09	12
	11:10	7
	11:11	7
Theophylline sodium, 1 cc. (1–1000)	11:14	5
	11:15	7
Theophylline sodium, 1 cc. (1–1000) (*cont'd*)	11:16	8
	11:17	15
	11:18	18
	11:19	19
	11:20	9
	11:21	9
	11:22	8
	11:23	6
New normal	11:26	7
Hydroxyethyl theophylline, 1 cc. (1–1000)	11:27	7
	11:28	11
	11:29	8
	11:30	8
	11:31	7
Aminophylline, 1 cc. (1–2000)	11:35	4
	11:36	7
	11:37	11
	11:38	14
	11:39	10
	11:40	4
Dihydroxypropyl theophylline, 1 cc. (1–2000)	11:44	12
	11:45	20
	11:45	18
	11:46	13
	11:47	13
	11:48	15
	11:48	8
	11:49	7
Theophylline, 1 cc. (1–2000)	11:52	10
	11:53	17
	11:54	20
	11:55	24
	11:56	16
	11:57	12
	11:58	10
	11:58	7
Theophylline glucoside, 1 cc. (1–2000)	12:00	7
	12:01	7
	12:02	15
	12:03	16
	12:04	15
	12:05	10
	12:06	7
	12:07	8
	12:08	7

Likewise over 50% of the aminophylline was precipitated in the intestinal fluids wherein the *p*H was less than 7.

Diuretic Acitivity.—The modified method for testing the diuretic activity of the drug dihydroxypropyl theophylline and other xanthine derivatives as developed by Lipschitz, Hadidian, and Kerpcsar was adopted in general (15).

Healthy albino rats weighing 130 to 240 Gm. were fasted for eighteen hours previous to the experiment, and were given the substances to be tested in proportion to their weight. All drugs to be tested were dissolved in isotonic sodium chloride solution at room temperature and administered by stomach tube. After the stomach tubing, the animals were placed in subgroups of four, in special metabolism cages and permitted to remain there without food or water for the duration of the experiment. At the end of a five-hour period the animals were taken out of the cages and the total volume of urine excreted by each group measured.

In the diuretic studies one hundred and twenty-four rats representing thirty-one subgroups were used in establishing the diuretic activity. Table III summarizes the comparable diuretic activity of dihydroxypropyl theophylline with urea and aminophylline. The data bear out the above statement that theophylline is an active diuretic in small doses but higher doses cause a decrease in diuretic activity as compared with the optimum lower dose. The dose for maximum diuretic activity for dihydroxypropyl theophylline determined by these experimental studies was found to be approximately twenty mg. per Kg.

EFFECT OF DIHYDROXYPROPYL THEOPHYLLINE ON BLOOD VESSELS

Oncometer studies in experimental animals show that there is a definite increase in organ volume following the administration of a xanthine primarily due to vasodilation coupled with an augmented cardiac output (16). The increased vascular effect experienced with theophylline and its derivatives is due to direct relaxation of the musculature of the vessel walls.

Experimentally the turtle's hind leg vessels allow a study of the peripheral action of various drugs upon the blood vessels. There is no influence of heart action in this setup as it is out of the circulation. Quantitative measurements of the outflow from the abdominal veins provide an index as to relative vascular effects.

The legs were perfused with Clarke's solution at room temperature. The solution was allowed to flow in by gravity from a reservoir, at a constant pressure of 12 centimeters of water pressure. As soon as the fluid escaping from the outflow tube was fairly free from blood, the outflow per minute was recorded. The data obtained are shown in Table IV.

Action of Dihydroxypropyl Theophylline on the Circulation of the Dog.—All parts of the circulatory system are affected in some way by the xanthines (17). A dog was anesthetized and prepared for the study of the action of dihydroxypropyl theophylline on the circulation.

Before the administration of any drugs, a normal blood pressure, pulse, and respiration were obtained. After the administration of each drug the action was recorded from the onset to a point where the pulse, respiration, and blood pressure returned to normal. The data in Table V bring forth the following points in connection with the circulatory action of dihydroxypropyl theophylline:

1. Increase in pulse rate which is in accordance with the fact that xanthines are direct myocardial stimulants.

2. No increase in respiration, indicating lack of central nervous system stimulation. This observation is further supported by observation of respiration of rats having received large doses of dihydroxypropyl theophylline.

3. Little change in blood pressure apparently resulting from the opposing actions of the direct myocardial stimulation and the peripheral vascular dilation.

In comparing the action of the xanthine derivatives caffeine and dihydroxypropyl theophylline, the well-known pharmacological fact of respiratory stimulation by caffeine was very evident but there was no stimulation by dihydroxypropl theophylline, although both stimulate the heart action to approximately the same degree.

Effect of Dihydroxypropyl Theophylline on the Isolated Heart.—The isolated mammalian heart offers an excellent means of studying the action of drugs upon the heart and coronary vessels without their effects being complicated by changes in the peripheral vascular bed.

The technique used for the perfusion of the isolated mammalian heart was a modified Langendorff (18) method.

Coronary Effect.—Having obtained dilatation of the vessels of the turtle hind leg vessels, it was expected that the coronary flow would be markedly increased. The data (Table VI) show that such a response was obtained. In comparing the response obtained from 1 cc. of 1–1000 solution of dihydroxypropyl theophylline with the same dose and concentration of aminophylline, nearly identical results were obtained with the coronary flow.

Rate of Amplitude.—When the same doses of each

TABLE V.—ACTION OF DIHYDROXYPROPYL THEOPHYLLINE ON THE CIRCULATION OF THE DOG

Drug	Pulse Rate	Respiration Rate	Blood Pressure, Mm.	Time, P. M.
Normal	200	36	110	2:45
	198	36	112	
	198	36	110	
	168	36	123	
	192	36	110	
	192	36	110	
	174	36	114	
	170	36	110	
Caffeine, 100 mg./dog	228	36	126	2:56
	270	48	126	
	270	54	125	
	264	54	120	
	264	48	114	
	264	54	104	
	264	48	108	
	264	48	112	
	240	48	114	
	222	36	114	3:00
	222	36	114	
	222	30	112	
	210	30	112	
	204	36	110	
	210	36	112	
Nitroglycerin, 1 cc. Spt./dog	198	36	118	3:08
	312	36	110	
	342	36	90	
	282	36	80	
	294	36	60	3:10
	294	36	70	
	300	30	80	
	318	36	90	
	276	36	94	
	264	36	98	
	240	36	100	
	216	36	112	
	200	36	114	3:15
Dihydroxypropyl theophylline, 100 mg./dog	180	36	110	
	210	36	112	
	240	36	110	
	270	36	108	
	258	36	106	
	240	36	108	3:18
	252	36	110	
	216	36	110	
	222	36	112	
	192	36	108	
	230	36	110	
	216	36	112	3:22

of the drugs aminophylline and dihydroxypropytheophylline were given, the increase in heart rate and amplitude of stroke was substantially greater with the aminophylline as shown in Table VI.

TABLE VI.—EFFECT OF DIHYDROXYPROPYL THEOPHYLLINE ON THE ISOLATED HEART

Drug	Rate	Amplitude of Stroke	Coronary Outflow
Normal	100	+	2
	100	+	2
	104	+	2
Theophylline, 1 cc. (1–1000)	116	+	3
	116	+	3
	120	+	3½
	116	+	3½
	114	+	2
	110	+	2
	104	+	2
Aminophylline, 1 cc. (1–1000)	152	+++	3
	150	+++	3
	152	+++	3
	145	+++	2½
	140	+++	2½
	130	++	2
	120	++	2
	110	+	2
	102	+	2
Dihydroxypropyl theophylline, 1 cc. (1–1000)	140	++	3
	140	++	3
	135	++	3
	132	+	2½
	121	+	2
	110	+	2
Theophylline sodium	145	+++	2½
	142	++	2½
	133	+	2
	126	+	2
	114	+	2
Theophylline glucoside, 1 cc. (1–1000)	122	+	2½
	120	+	2½
	114	+	2
	106	+	2
Hydroxyethyl theophylline, 1 cc. (1–1000)	126	+	2½
	122	+	2½
	118	+	2
	109	+	2
Dihydroxypropyl theophylline, 1 cc. (1–500)	163	+++	3
	155	+++	3
	151	++	2¾
	145	++	2½
	140	++	2½
	132	++	2
	121	+	2
	110	+	2

Action of Dihydroxypropyl Theophylline on the Bronchiolar Muscles.—Herrmann and Aynesworth (19) have found that theophylline and, particularly, aminophylline are of benefit in the treatment of bronchial asthma and are of special value in patients refractory in epinephrine. Favorable responses in bronchial asthma have also been reported by others (20) with the use of aminophylline. This use is now accepted by the Council on Pharmacy and Chemistry of the American Medical Association.

For the purpose of determining the action of dihydroxypropyl theophylline on the bronchial musculature, the isolated lungs of the guinea pig were perfused with Clarke's solution.

A study of the data disclosed that from a normal flow of the perfusion liquid a substantial increase was obtained by the action of dihydroxypropyl theophylline. Likewise after the administration of histamine, which resulted in constriction of the bronchiolar muscles, the administration of dihydroxypropyl theophylline again brought a relaxation of the muscles as indicated by the flow. In comparing the action of dihydroxypropyl theophylline with aminophylline, it was noted that an equal dosage of dihydroxypropyl theophylline produced the greater response.

CLINICAL STUDIES

Aminophylline is indicated chiefly in the therapy of coronary disease and its value in such conditions is fairly well established. To compare dihydroxypropyl theophylline with aminophylline, patients with coronary disease were selected and given the new drug in the same dosage as that indicated for aminophylline, namely, 1½ gr. A few of these patients were seized with complicated cardiac disease and received therapy in addition to dihydroxypropyl theophylline. These cases do not present a clear picture of efficacy because of the other therapy employed. In the cases in which the new drug was the chief therapeutic agent employed, all patients showed excellent improvement. Dihydroxypropyl theophylline appeared to equal aminophylline in efficacy and there were no complaints of gastric upset or intolerance to the drug. However, it will require a much larger series of cases to determine the new drug's true status of efficacy when compared with aminophylline. The clinical study is being continued and will be reported in full at a later date.

SUMMARY

1. The preparation, identification, and properties are described for dihydroxypropyl theophylline, a stable white crystalline solid, forming neutral aqueous solutions stable in the digestive fluids of the body.

2. Toxicity studies revealed the drug to be relatively nontoxic orally or intravenously. Chronic toxicity studies likewise revealed no histological changes in the kidneys when rats were given large daily doses over a thirty-day period.

3. It was found from the pharmacological tests that dihydroxypropyl theophylline possessed the following properties which are desirable for clinical use:

(*a*) Diuresis, as demonstrated on rats.

(*b*) Direct myocardial stimulation as shown on isolated heart of the rabbit and on the intact dog.

(*c*) Dilatation of coronary and other circulatory vessels as shown on the turtle and rabbit.

(*d*) Dilatation of bronchiolar vessels as shown on the lungs of the guinea pig.

(*e*) Low toxicity when given by mouth or intravenously to rats.

(*f*) Stability in gastric and intestinal fluids, a characteristic not possessed by theophylline derivatives now in use.

4. Dihydroxypropyl theophylline, being a definite chemical compound, is probably absorbed unchanged for the exertion of its pharmacological action, an event which provides definite advantages for clinical studies not present with theophylline derivatives now in use.

5. Most theophylline derivatives now in use apparently decompose, leaving basic theophylline in digestive fluids to perform its pharmacological and therapeutic effect.

REFERENCES

(1) "Council on Pharmacy and Chemistry," *J. Am. Med. Assoc.*, **94**, 17, 1306(1930).
(2) Amep, G. V., *Arch. exptl. Path. Pharmakol.*, **138**, 118 (1928).
(3) Heathcote, R. St. A., *J. Pharmacol.* **16**, 327(1920–21).
(4) Smith, F. M., Miller, G. H., and Graber, V. C., *J. Clin. Investigation*, **2**, 157(1926).
(5) Askanazay, S., *Deut. Arch. klin. Med.*, **56**, 206(1926).
(6) Breuer, R., *Münch. med. Wochschr.*, **49**, 1604(1902).
(7) Theohari, A., *Arch. maladies coeur vaisseaux*, **16**, 481 (1923).
(8) Smith, F. M., Graber, V. C., and Miller, G. H., *Trans. Sec. Pharmacol. Therap., J. Am. Med. Assoc.*, **2**, 171(1926).
(9) Wood, C. W., and Osol, A., "United States Dispensatory," ed. 23, J. B. Lippincott Co., Philadelphia, 1943, p. 1126.
(10) Boyer, N. H., and Green, H. P., *Am. Heart J.*, **21**, 199(1941).
(11) D. R. P. 191106, patented in the German Reich, Aug. 9, 1906.
(12) D. R. P. 281008, patented in the German Reich, Aug. 14, 1913.
(13) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," The MacMillan Company, New York, 1941, p. 280.
(14) Adams, G. A., *J. Am. Med. Assoc.*, **123**, 1113(1943).
(15) Lipschitz, W. L., Hadidian, Z., and Kerpesar, A., *J. Pharmacol.*, **49**, 2, 97(1943).
(16) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," 1941, p. 278.
(17) *Ibid.*, p. 279.
(18) Gross, E. G., "Laboratory Manual of Pharmacology for Medical Student Instruction," Mimeographed and bound in State University of Iowa, Department of Pharmacology, Iowa City, Iowa, 1945.
(19) Herrmann, G., and Aynesworth, M. B., *J. Lab. Clin. Med.*, **23**, 135–48(1937).
(20) Boyer, N. H., *Journal American Medical Association* **122**, 306(1943).

A Chemical Investigation of Celastrus Paniculata Willd.*

By N. K. BASU and P. R. PABRAI

Two alkaloids, Celastrine and Paniculatine, have been isolated from *Celastrus paniculata* Willd. and their physical and chemical properties described.

THE SEEDS of *Celastrus paniculata* (indigenously known as Malkangni), belonging to the natural order Celastraceae, are used as a remedy for rheumatism, gout, paralysis, and leprosy and are supposed to have the property of stimulating the intellect and sharpening the memory.

Presence of an alkaloid is mentioned by Wehmer (1) and Chopra (2). Chopra (2) mentions the presence of a glucoside and coloring matter as well in the seeds. Physical and chemical constants of the oil from seeds have been studied in detail by Kumaraswamy and Manjunath (3) and by Gunde and Hilditch (4). Kumaraswamy and Manjunath (3) have also obtained a phytosterol (m. p. 136°) and a nonnitrogenous substance from the unsaponifiable fraction of the oil while Warsi (5) reports a different sterol from the same fraction and the presence of a a bright orange-red coloring matter (2 per cent) in the oil.

Since no systematic work had been directed toward the isolation of the alkaloid reported in the drug, it appeared desirable to investigate it further. Two alkaloids were isolated from the oil-cake, but none were found in the oil expressed from the seeds. One alkaloid, which we named "Celastrine," was obtained in a pure crystalline state (m. p. 260°, with decomposition, and molecular formula, $C_{19}H_{25}NO_3$) but the other, which we named "Paniculatine," could not be purified.

EXPERIMENTAL

Isolation of the Alkaloid.—Twenty-five grams each of the powdered whole seeds and the oil-cake, obtained after expression of the oil, were extracted

* Received May 27, 1946, from the Department of Pharmaceutics, Benares Hindu University, Benares, India.

separately with different solvents. It was discovered that the maximum quantity of alkaloids was obtained by percolation or extraction in a Soxhlet apparatus with alcohol and that the oil-cake contained about double (0.088%) the amount of alkaloid found in the whole seeds. The fact that the oil does not contain any alkaloid was substantiated by shaking a portion of it with 1% HCl, testing the acid extract for alkaloid, and obtaining negative results. Accordingly, in all subsequent experiments the oil-cake was used for extraction of the alkaloids.

Four kilograms of the oil-cake was extracted in a Soxhlet apparatus with 95% alcohol. The solvent was removed by distillation until the residual mass was syrupy in consistency. It was then transferred to a separator, and the oily liquid was shaken with successive portions of 1% HCl to extract the alkaloid. The acid extract, after being filtered, was shaken repeatedly with chloroform until the coloring matter was removed completely, each chloroform extract being washed three times with 1% HCl. The chloroform extract, even after the third washing, gave a distinctly positive test for alkaloid. It was marked "x" and preserved for further investigation.

The decolorized acid extract was filtered leaving a light brown sticky resinous mass on the filter paper. This material was found to be soluble in alcohol and in dilute alkalis, sparingly soluble in ether, and insoluble in chloroform and dilute mineral acids. The filtrate was made alkaline with dilute ammonia and the liberated alkaloid extracted by shaking repeatedly with chloroform until complete extraction of alkaloid was effected. Each chloroform extract was washed with the same sample of water contained in another separator. The chloroform was distilled off and the residue, which was very resinous, was shaken with 1% HCl and the alkaloid re-extracted by the process outlined above. The crude alkaloidal residue, thus obtained, weighed 1.4 Gm.

Purification of the Crude Alkaloid.—The crude alkaloidal residue was dissolved in a large excess of chloroform, the solution was vigorously shaken for one hour with animal charcoal and kept overnight. It was then filtered, the solvent was distilled off leaving a white crystalline mass, embedded in a little of the resinous matter. This was then shaken repeatedly with ether which dissolved the alkaloid and left the resinous mass behind. When the ether was allowed to evaporate spontaneously, long needle-shaped crystals of the alkaloid, surrounded by a ring of the resinous matter, were obtained. They were washed several times with a few drops of alcohol, and from the alcoholic washings another crop of crystals was obtained. They were finally crystallized from chloroform and dried. The weight of the pure base thus obtained was 0.0611 Gm. The alkaloid melted with decomposition at 260°. It was found to be soluble in alcohol, ether, chloroform, and benzene but insoluble in water and in petroleum ether.

The following derivatives were prepared: (*a*) the aurichloride, which melted with decomposition at 120–122°; (*b*) the platinichloride, which melted with decomposition at 166°; and (*c*) the sulfate, having a melting point above 300°.

On submitting the base to combustion, the following results were obtained:

Carbon	72.51%
Hydrogen	7.94%
Nitrogen	4.44%
Oxygen (by difference)	15.11%

Its empirical formula, therefore, is $C_{16}H_{25}NO_3$ and the empirical weight 315.

The molecular weight of the alkaloid, determined by the cryoscopic method, using benzene as solvent, is 319.9 and that obtained by ignition of the alkaloidal platinichloride is 312.5, assuming the base to be monoacidic. The two values compare very closely and also with the empirical weight of the compound, so the molecular formula of the alkaloid is $C_{19}H_{25}NO_3$. We named this new alkaloid "Celastrine."

Isolation of Alkaloid from Chloroform Solution "x."—The chloroform was removed by distillation leaving a dark brownish residue which was extracted by shaking with 1% HCl. The mixed acid extract was filtered, rendered alkaline with dilute ammonia and the liberated alkaloid was extracted by shaking with chloroform. The solvent was removed by distillation and the crude residue weighed (0.0252 Gm.). No further purification could be attempted since the quantity obtained was very small, but its aurichloride, which decomposes at 140° was prepared. The name "Paniculatine" was given to this alkaloid.

SUMMARY

1. Two alkaloids, Celastrine and Paniculatine, have been isolated from the oil-cake of *Celastrus paniculata;* the former was obtained in a pure crystalline form having the molecular formula, $C_{19}H_{25}NO_3$ and melting with decomposition at 260° while the latter could be obtained only in a crude form.

2. Some derivatives of each alkaloid have been prepared.

3. The hydrochloride of celastrine is insoluble in chloroform while that of paniculatine is not.

4. A resinous body has also been detected.

REFERENCES

(1) Wehmer, Karl, "Die Pflanzenstoffe," G. Fischer, Jena, Germany, 1931, p. 717.
(2) Chopra, R. N., "Indigenous Drugs of India," Art Press, Calcutta, India, 1933, p. 473.
(3) Kumaraswamy, O. N., and Manjunath, B. L., *J. Ind. Chem. Soc.*, **13**, 353(1936).
(4) Gunde, B. G., and Hilditch, T. P., *J. Chem. Soc.*, **1938**, 1980.
(5) Warsi, S. A., *Current Science*, March, 1940.

A Chemical Investigation of Sphaeranthus indicus Linn.*

By N. K. BASU and P. P. LAMSAL

An alkaloid, Spaeranthine, has been isolated from *Sphaeranthus indicus*. A volatile oil, obtained by steam distillation of the fresh plant, has also been obtained. The isolation and purification of these two principles are described and their physical and chemical properties reported.

SPHAERANTHUS INDICUS is an annual herb of 1 to 2 feet in height, belonging to the natural order of *Compositae*. The plant is distributed throughout India and Ceylon at elevations up to 5000 feet. It also grows in Malaya, China, Africa, and Australia and is commonly found in rice fields and in marshy places. The drug has been used as a digestive tonic and antiseptic.

Regarding the chemical constituents of the plant, Dymock, *et al.* (1) reports the presence of an ether soluble alkaloid named "Sphaeranthine" and of a viscid deep sheery colored water-soluble oil.

An alkaloid, Sphaeranthine, was isolated in a pure form, having the molecular formula, $C_{13}H_{19}NO_3$, and melting at 166–168° with decompositon. Several derivatives have also been prepared. The physical and chemical constants of an essential oil, obtained by steam distillation of the fresh flowering plants, have also been determined.

EXPERIMENTAL

Preliminary experiments with small quantities of drug soon established that the alkaloid is best isolated by continuous extraction with hot alcohol and that prolonged contact with alkalis should be avoided.

The alkaloid is precipitated from an acid solution by Mayer's, Dragendorff's, Wagner's, and other alkaloidal reagents. Dragendorff's reagent was found to be most effective for this purpose.

Isolation of the Alkaloid.—Four kilograms of the coarsely powdered drug was extracted with hot alcohol in a Soxhlet apparatus. The extract was concentrated by distillation to a syrupy consistency and the concentrate thus obtained was poured gradually into warm 1% HCl and the mixture shaken thoroughly in a mechanical shaker. The acid mixture was filtered and the residue washed with 1% HCl until the acid washing did not give any test for the alkaloid. The acid extracts were combined and shaken with successive portions of chloroform until the coloring matter was removed completely. The acid extract was made alkaline with dilute ammonia and the liberated alkaloid was extracted completely with chloroform. This chloroform extract was washed with water, then dried over anhydrous magnesium sulfate, and finally filtered. The chloroform was removed by distillation and the remaining brownish alkaloidal residue was purified.

Purification.—The crude alkaloid was dissolved in a few mils of chloroform, ether was added drop by drop and a brown mass separated at once. The supernatant liquid was decanted, a little gasoline was added, and a colored precipitate formed. The supernatant liquid was decanted, gasoline in excess added, and a light-colored substance precipitated, which was separated and dried in a vacuum dessicator. Further attempts to crystallize the base were not successful. The melting point of the base as finally determined was 166–68° with decomposition. The alkaloid weighed 0.1120 Gm.

The result of the micro-combustion of the base was as follows:

Carbon	58.41 %
Hydrogen	7.239%
Nitrogen	5.252%
Oxygen (by difference)	29.099%

The empirical formula calculated from the above data was $C_{13}H_{19}NO_3$. The molecular weight of the alkaloid as determined by the cryoscopic method, using benzene as solvent, was found to be 250 and the molecular weight obtained by ignition of the platinichloride derivative was 266.02. Thus the molecular formula of the base, assuming it to be monoacidic, is $C_{13}H_{16}NO_3$. The molecular weight calculated from the above formula, therefore, is 269. The melting points of the derivatives of the alkaloid are given below:

Hydrochloride	m. p. 158–60° (decomp.)
Sulfate	decomposed at 185°
Tartrate	m. p. 132° (decomp.)
Oxalate	m. p. 110–11° (decomp.)
Picrate	m. p. 154–56° (decomp.)
Platinichloride	decomposes at 196–98°

Essential Oil.—By steam distillation, on a small laboratory scale, of the fresh flowering plants, 25 mils of oil was collected. After dehydration over anhydrous magnesium sulfate, the following physical and chemical constants were determined:

* Received May 27, 1946, from the Department of Pharmaceutics, Benares Hindu University, Benares, India.

Specific gravity at 30°	0.9419
Refractive index at 20°	1.1512
Optical rotation	nil
Acid value	2.4030
Ester value	47.80
Ester value (after acetylation)	74.15

SUMMARY

1. A new alkaloid, Sphaeranthine, of molecular formula, $C_{13}H_{19}NO_5$ and m. p. 166–68°, has been isolated from the plant, *S. indicus*.

2. An essential oil has also been obtained and its physical and chemical constants have been determined.

REFERENCE

(1) Dymock, William, Warden, C. J., and Hooper, David, "Pharmacographica Indica," Vol. II, K. Paul, Trench, and Trübner, London, 1893, p. 257.

Hydrophilic Acid Ointments for the Debridement of Burns*†

By ALBERT M. MATTOCKS and WILBUR A. LAZIER‡

A number of readily wetted powders for the formation of acid-containing ointments are described. These ointments were formulated for use in the chemical debridements of burns. Several were found to be as effective as pyruvic acid-starch paste mixtures originally employed. Methods of preparation, assay procedures, and data on the stability of several ointments are reported.

THE ADVENT of the sulfa drugs and other powerful antibiotics has made possible an entirely new approach to the treatment of deep burns, whether caused by fire or by chemical agents. However, the removal of necrotic tissue from the site presents a special problem. Mechanical debridement by scrubbing or surgery causes much pain with increased danger from shock, and may actually damage or remove residual islands of epithilial cells capable of regenerating new tissue.

Chemical debridement was investigated by G. Connor and S. C. Harvey of Yale University (1) who found that jellies containing organic acids such as pyruvic or succinic acids cause removal of slough in a single tough pellicle, leaving a clean granulating surface. This work was confirmed by M. B. Sulzberger, R. L. Baer, and A. Kanof, and by J. Wexler and G. Rosenfeld (2). Harvey and Connor used pyruvic acid-cornstarch paste which had a *p*H of 1.9, but they pointed out that the composition of the ointment was probably not optimum.

They also suggested that the optimum *p*H and molarity of the pastes probably varied with the acid used. Since both the acid and vehicle of the pyruvic acid-starch paste were known to be unstable, a search for more stable acids and vehicles was begun under the auspices of OSRD by the CMR group at Cornell Medical College in New York. Tartaric, succinic, phosphoric, and pyruvic acids were found to be effective, and many vehicles such as carbowax, carboxymethylcellulose, methylcellulose, K-Y jelly, triglycerol cream, starch-glycerin, bentonite, alumina, polyvinyl alcohol, polyvinyl acetate, and several natural gums were investigated. Methylcellulose-sorbitol gels were found to be as effective as starch pastes by Rosenfeld and Sulzberger, *et al.*

In April, 1945, experimental work was begun on the problem of preparing dry, stable

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‡ In addition to collaboration by the Cornell Medical College group, the authors wish to acknowledge the valued assistance of Maxine Brogden and Olivia Hutchison who participated in this program.

powders capable of being mixed with water to form gels suitable for the debridement of burns. Previous formulations had been bulky due to the large content of water, and were susceptible to microbial attack unless kept under aseptic conditions. On account of the copious quantities of gel needed for treatment, it seemed imperative that some means be found for reducing the bulk of the material to be stored. Quick wetting of the powder to form an ointment of the proper consistency was recognized as one of the problems to be solved.

Methylcellulose (high viscosity), hydroxyethylcellulose (medium and high viscosity), and tragacanth were investigated as possible bases for the dry mixtures. Methylcellulose was found to be most suitable, forming readily wetted mixtures and permitting ointments of widely different *p*H to be prepared. Tragacanth mixtures were invariably slow in wetting, and hydroxyethylcellulose mixtures apparently inhibited the ionization of acids in the finished gels. Preliminary work demonstrated that Xynomine paste (Onyx Chemical Corp.) and Arlex (85% sorbitol solution—Atlas Powder Co.) were satisfactory for the methylcellulose mixtures. Arlex was the better of the two, for Xynomine paste imparted color to the mixtures and caused incorporation of much air into the gels formed.

Tartaric, pyruvic, and phosphoric acids were investigated as chemical agents. Tartaric acid was reported by the Cornell Medical Group to be less effective in debridement than pyruvic acid. Work with phosphoric acid was emphasized for the reason that it was believed to be more stable toward heat and storage than pyruvic acid.

Several methods of mixing ingredients of the debridement formulas were investigated. It was found that the addition of Arlex and phosphoric acid to powdered methylcellulose followed by regrinding, produced powders that could be wetted in less than two minutes. Mixtures containing various concentrations of acids were prepared by this method and forwarded to the Cornell Group for testing. Assays of these mixtures indicated that they were not always uniform in composition. An improvement in mixing procedure was developed whereby the acid in acetone solution was added to the methylcellulose. The solvent was then evaporated, the wetting agent was incorporated, and the mixture was ground to a fine powder. Several mixtures made by this procedure were sent to Cornell for testing on burns. These powders were not easily prepared without loss of acid and wetting agent during the milling process, and they were quite bulky.

Further improvement of the mixing procedure led to the discovery that the addition of methylcellulose with stirring to an aqueous solution of acid and wetting agent produced clear homogeneous gels after stirring for several hours. These gels were spread out on sheets of glass in layers about 0.5 cm. thick and dried in an oven at 75°. The films thus produced where ground in a micropulverizer. The finely powdered products wetted in twenty to thirty seconds with the production of clear, lump-free gels of uniform composition. These products had an added advantage in that they possessed much less bulk than the methylcellulose mixtures prepared by the first two methods.

Storage tests of methylcellulose-phosphoric acid mixtures for two weeks at 50° resulted in no observable changes in physical properties or in effectiveness of debridement. Methylcellulose and hydroxyethylcellulose preparations were stored at 50° and 100% relative humidity without showing appreciable changes in moisture or acid content.

Methylcellulose mixtures and hydroxyethylcellulose mixtures containing acid but no wetting agent were prepared and sent to the Cornell Medical Group for testing on burns. No adverse effects due to presence of wetting agents could be detected.

The mixtures of methylcellulose, phosphoric acid, and sorbitol prepared by the third procedure outlined above were considered to fulfill the physical requirements for practical debriding agents. These mixtures proved to be non-bulky, they formed smooth uniform gels quickly by the addition of water, and they served as stable carriers of acid in various concentrations without inhibiting ionization.

EXPERIMENTAL

In all experiments the tartaric acid used was finely pulverized c. p. tartaric acid, the pyruvic acid was c. p. grade, and the phosphoric acid was c. p. 85% material.

Soluble Starch Mixtures.—Studies of soluble starch with wetting agents produced no mixtures from which the starch would not separate after mixing with water.

then evaporating the solvent. Formulas in Tables II and III represent amounts used with 100 Gm. of hydroxyethylcellulose. (A sample of each mixture was forwarded to the Cornell Group.) Wetting was rapid for mixtures containing wetting agents. Debridement has been shown to be most complete with mixtures of *p*H less than 2, but it is evident from the tables that a *p*H below 2 was not obtainable with this vehicle even though relatively high concentrations of acid were used.

TABLE I.—TRAGACANTH MIXTURES

Sample No.	Acid Used	Gm. Acid	Wetting Agent	Gm. W. A.	Solids Gm./100 Cc.	Wetting Time, Min.	*p*H
717	Tartaric	23.3	Arlex	10	9.9	10	2.70
721A	Tartaric	28.0	Arlex	18	20.0	6	2.60
727A	Pyruvic	9.5	Arlacel C	25	13.3	7	2.50

TABLE II.—HIGH VISCOSITY HYDROXYETHYLCELLULOSE MIXTURES

Sample No.	Acid Used	Gm. Acid	Wetting Agent	Gm. W. A.	Solids Gm./100 Cc.	Wetting Time, Min.	*p*H
732A	Pyruvic	4.85	Arlacel C	25	33.3	1.5	2.50
732B	Pyruvic	4.85	None	..	33.3	4	2.60
734A	Phosphoric	4.94	Arlacel C	25	33.3	2	2.65
734B	Phosphoric	4.94	None	..	33.3	4	2.60
734C	Pyruvic	8.89	Arlacel C	25	33.3	2	2.30
734D	Pyruvic	8.89	None	..	33.3	4	2.25
742	Pyruvic	8.89	None	..	16.6	4	2.28
743	Phosphoric	3.93	None	..	16.6	4	2.52

TABLE III.—MEDIUM VISCOSITY HYDROXYETHYLCELLULOSE MIXTURES

Sample No.	Acid Used	Gm. Acid	Wetting Agent	Gm. W. A.	Solids Gm./100 Cc.	Wetting Time, Min.	*p*H
727B	Pyruvic	4.85	Arlacel C	25	33.3	1.5	2.50
738	Phosphoric	4.73	None	..	33.3	5	2.58
739	Pyruvic	8.89	None	..	33.3	3	2.25
740	Phosphoric	3.90	Arlacel C	25	33.3	2	2.53
741	Pyruvic	8.89	Arlacel C	25	33.3	2	2.20

Tragacanth Mixtures.—Tests with tragacanth and wetting agents showed Arlex and Arlacel C (Atlas Powder Co.) to be the most suitable wetting agents for this base, but all mixtures wetted slowly. Mechanical incorporation of ingredients followed by sieving served to mix these samples. Formulas listed in Table I represent quantities of ingredients incorporated with 100 Gm. of tragacanth. The column headed "Solids" indicates the number of parts by weight of finished powder to be added to one hundred parts of water in order to form the ointment. (A sample of each mixture was forwarded to Cornell Medical College.) All mixtures wetted slowly to form gels containing much incorporated air. Tragacanth was thus considered an inferior base.

Hydroxyethylcellulose Mixtures.—Preliminary tests showed Arlacel C to be a suitable wetting agent for hydroxyethylcellulose of both high and medium viscosity. All the mixtures were prepared by adding the wetting agent and/or acid in acetone solution to commercial powdered hydroxyethylcellulose and

Methylcellulose Mixtures.—Mixtures of methylcellulose and acids along with various wetting agents were prepared. Many of the mixtures were found to wet readily, but only those that formed homogeneous ointments within two minutes were considered satisfactory. Concentrations of acids were based on suggestions of the Cornell Medical Group and on previously reported gels. Some limitations were imposed by milling procedures, for damp powders could not be ground without considerable loss of ingredients. It was found that the Arlex mixtures were superior to those containing the other wetting agents used; the others produced colored mixtures or caused incorporation of much air into the finished product. Three procedures were used for mixing the powders: (a) Powdered methylcellulose was mixed with wetting agent and acid, and the mixture was passed through a pulverizer. (b) An acetone solution of the acid was added to powdered methylcellulose, and the solvent was allowed to evaporate; finally, the wetting agent was incorporated, and the mixture was ground in a pulverizer. (c) Methyl-

cellulose in commercial form was added to an aqueous solution of wetting agent and acid, and stirring was continued until a homogeneous ointment was formed. The mass was spread on glass plates in 0.5-cm. layers and dried in an oven at 75° for twenty-four hours, and the material was then pulverized. Since the moisture content of finished powder was somewhat variable, the amount of water to add for ointment preparation was calculated from the acid content shown by analysis. In Tables IV, V, and VI, the methods used for mixing are indicated by numbers corresponding to the above descriptions.

The mixtures containing Xynomine and Arlacel were colored, and their ointments contained many air bubbles due to the foaming properties of these wetting agents. The Arlex mixture wetted satisfactorily to form a clear gel. It was bulky, however, being prepared by Procedure 2.

Acid Concentration of Mixtures.—Previously reported tests indicated that gels with *p*H values between 1.8 and 2.0 were most effective in debridement, although it was believed that the optimum *p*H varied with the acid and vehicle used. Samples of each type of mixture with various acid concentrations were subjected to test, and results of *in vivo* tests made by the Cornell Medical Group (3) served as criteria. For methylcellulose gels, 1% phosphoric or pyruvic acid appeared to be near optimum concentration. Greater detail regarding the rela-

TABLE IV.—METHYLCELLULOSE-PHOSPHORIC ACID MIXTURES

Sample No.	Gm. Acid	Wetting Agent	Gm. W. A.	Solids Gm./100 Cc.	Wetting Time, Min.	*p*H	Method of Preparation
737	6.67	Arlex	20	13.3	2	1.88	2
744	6.67	Arlex	20	10	1	2.00	2
745	10.00	Arlex	20	10	1	1.79	2
746	16.70	Arlex	20	10	1	1.60	2
747	20.00	Arlex	20	10	1	1.58	2
756	6.67	Arlex	20	10	1	1.98	2
757	10.00	Arlex	20	10	1	1.88	2
758	13.30	Arlex	20	10	1	1.79	2
759	10.00	None	..	10	6	1.89	2
769	10.00	Arlex	20	9.2	1	1.92	3
770	6.67	Arlex	20	8.7	1	2.05	3
772	13.30	Arlex	20	9.2	1	1.85	3

TABLE V.—METHYLCELLULOSE-TARTARIC ACID MIXTURES

Sample No.	Gm. Acid	Wetting Agent	Gm. W. A.	Solids Gm./100 Cc.	Wetting Time, Min.	*p*H	Method of Preparation
77A	14.2	Xynomine	20	13.3	2	1.80	1
77B	14.2	Arlex	20	13.3	2	1.80	1
77C	7.6	Arlex	20	24.0	2	1.90	1
712B	17.2	Xynomine	20	13.3	2	1.99	1
714A	17.2	Arlex	20	13.3	2	1.99	1
718	6.0	Tragacanth	10	13.3	20	2.35	1
721C	27.9	None	..	13.3	Not satisfactory		1

TABLE VI.—METHYLCELLULOSE-PYRUVIC ACID MIXTURES

Sample No.	Gm. Acid	Wetting Agent	Gm. W. A.	Solids Gm./100 Cc.	Wetting Time, Min.	*p*H	Method of Preparation
720A	9.5	Xynomine	20	13.3	2	1.87	1
725A	9.5	Intral-229 Synthetic Chem.	20	13.3	6	1.88	2
729B	13.2	None	..	13.3	6	1.90	2
731	13.2	None	..	13.3	6	2.00	2
736	12.3	Arlex	20	13.3	2	2.28	2
76A	16.7	Xynomine	68	11.0	1	2.05	1

All of these mixtures that contained Arlex wetted readily and formed smooth gels, but the last three were superior in wetting qualities.

All but the last two of these wetted with satisfactory rapidity. The mixtures containing Xynomine were dark in color (due to the wetting agent)

tion between effectiveness and acid concentration will, no doubt, be included in the reports of the Cornell Medical Group.

Density of Mixtures.—Methylcellulose and hydroxyethylcellulose mixtures prepared by Methods 2 and 3 were compared as to bulkiness by weighing equal volumes of powders. The mixtures of procedure 3 showed a considerable reduction in bulk (Table VII).

TABLE VII.—DENSITY OF METHYLCELLULOSE AND HYDROXYETHYLCELLULOSE MIXTURES

Sample No.	Mixing Procedure	Bulk Gm./100 Cc.	Gm. Ointment formed from 100 Cc. Powder
737	2	6.83	58.0
769	3	29.95	356.0
745	2	6.80	74.8
742	2	14.92	104.4
736	2	5.60	47.6
741	2	17.01	68.0
770	3	47.18	589.0
772	3	44.88	537.9

Assays of Finished Mixtures.—Many of the mixtures prepared were assayed by titration with tenth normal sodium hydroxide in an effort to find the acid contents. Results on samples prepared by Procedures 1 and 2 were generally inconsistent, indicating that the mixtures were not uniform but the methylcellulose-Arlex-phosphoric acid mixtures prepared by method 3 gave reproducible values on titration.

Storage Tests.—Three hundred-gram samples of mixtures No. 756, 757, 758, and 759 in cardboard cannisters were placed in an oven at 50° for ten days. At the end of this time there were no changes in physical characteristics, and the *p*H values of the ointments formed by them were within 0.08 unit of the values before storage. Three hundred-gram samples of the same mixtures were stored in cardboard cannisters at room temperature for ten days, and no changes were noted. These storage samples were then tested by the Cornell Medical College Group who found that no changes in effectiveness had taken place. One-gram samples of mixtures No. 714A, 721A, and 725A were placed in open containers in an oven at 50° with 100% relative humidity. No gain in weight in any of the samples was noted.

Results of in Vivo Tests.—The group under the direction of M. B. Sulzberger at Cornell Medical College made all *in vivo* tests of the mixtures described. Their complete results will be reported separately, but private communications indicated that samples 720A, 729B, 736, 737, 741, 742, and 745 were approximately as effective as pyruvic acid-starch paste in debridement. Samples after two weeks' aging at 50° showed no loss of effectiveness.

SUMMARY

Stable, dry powders were prepared that were rapidly wetted with water to form acid-containing ointments as effective as pyruvic acid-starch paste in chemical debridement of burns.

REFERENCES

(1) Connor, G., and Harvey, S. C., *Ann. Surg.*, **120**, 362 (1944).
(2) Personal Communications.
(3) Sulzberger, M. B., Kanof, A., and Baer, R. L., *Ann. Surg.*, in press.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

d-Pulegone
Kynurenic acid
Crocin
Picrocrocin
Phloron
2-Acetaminoquinone
2,6-Dimethoxyquinone
Hydrophloron
Trilactic acid
4,4′-Diamidino diphenyl sulfone
4,4′-Diamidino diphenyl ether
9-(2-Diamylamine-1-hydroxyethyl)-1,2,3,4-tetrahydrophenanthrene
N-Methyl thiourea
N,N-Dimethyl thiourea
N,N′-Dimethyl thiourea
N,N-Diethyl thiourea
Methyl isothiocyanate
Ethyl isothiocyanate
Adoninulose
Chloralose
Methyl ethyl ether

The Gastrointestinal Absorption and Destruction of Penicillin*

By VICTOR P. SEEBERG, PAUL L. ILLG, and DORIS JANE BROWN
With the Technical Assistance of MARY L. WICKERT

Clinical reports indicating oral penicillin to be approximately one-fifth as effective per unit administered as parenteral penicillin were confirmed by measurements of urinary excretion in cats. To explain this large difference, a detailed pharmacological study of the sites of maximal and minimal gastrointestinal absorption and destruction of penicillin was made. Organs in order of increasing absorption are: esophagus, colon, stomach and small intestine. Organs in order of increasing destruction are: duodenum, jejunum, ileum, and colon. Absorption from the ligated duodenum, when sufficient time is allowed, yields urinary recoveries comparable to parenteral administration. Thus the inefficiency of an oral dose can be attributed primarily to incomplete absorption in the upper intestinal tract and subsequent destruction in the lower regions. Research directed toward improving the efficiency of an oral dose should be along lines to increase the rate of absorption of penicillin in the upper intestine.

THE WIDE field of therapeutic application of penicillin has prompted numerous searches for a practical method of oral administration to overcome the disadvantages of frequent parenteral administrations now largely employed in a course of treatment. Several excellent reviews of the problem have appeared in the literature by Cutting *et al.* (1), Finland, *et al.* (2), Bunn, *et al.* (3), and others. Most of these investigators are in general agreement that the oral dosage requirements are four or five times the parenteral in order to obtain equal serum concentrations of the drug and comparable therapeutic effectiveness. This approximate oral/parenteral ratio is also substantiated by measurements of total urinary excretion of penicillin. For example, Free and coworkers (4), found that in human beings, an average of 60 per cent of an administered dose could be recovered from the urine after intramuscular administration, while an average of 14 per cent was found after oral administration. It seemed to us that a better understanding of the reasons for the inefficiency of an oral dose was necessary before any logical methods could be applied to increase the efficiency.

Early reports indicated the most important destructive influence on orally administered penicillin to be the acidity of the stomach (5, 6). Following this, many optimistic clinical reports appeared in the literature on the use of agents to protect the penicillin against gastric destruction such as oils (7, 8), antacids (9–12), and enteric coatings (13). However, later and better controlled studies have shown that the destructive influence of gastric acidity is at the most only a minimal factor in accounting for the major loss of an oral dose. For example, Free found in his clinical series that when the oral dose was administered with an antacid to neutralize gastric acidity, or when the oral dose was administered by means of a duodenal tube, the average penicillin recovery from the urine was about 23 per cent as compared to 14 per cent recovery after oral administration without protection. Finland reported somewhat higher and more sustained serum concentrations in achlorhydric individuals as compared to normal individuals but only slight evidence of increased absorption by the use of gastric protectives in normal subjects. McDermott, *et al.* (14, 15), were unable to show a significant increase in penicillin serum concentrations or urinary excretion by the use of gastric protectives.

Free proposed three other possible causes for the major loss of an oral dose. First, since penicillin absorption from the gastrointestinal tract results in a more uniform rate of entry into the circulation than from a parenteral injection, it may allow for a longer

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exposure of penicillin to destructive influences in the body proper. Second, orally administered penicillin enters the portal circulation and is more completely exposed to the metabolic activities of the liver. Third, penicillinases derived from intestinal bacteria may inactivate a large portion of an oral dose before it can be absorbed. Cutting (1) has also suggested that possibly poor absorption and subsequent destruction by lower intestinal penicillinases are of importance, and a recent clinical report by McDermott, *et al.* (16) gives experimental evidence to establish this as fact.

The first proposal of Free appeared to us a very difficult one to evaluate directly, and also one which would not be likely to explain the large loss encountered. As to the second, Cutting, *et. al.* (17), obtained evidence against hepatic destruction when similar blood levels were reached and maintained in rabbits following intravenous injection into ear vein and portal vein. We therefore became interested in evaluating the third proposed explanation, the possibility of poor intestinal absorption and subsequent intestinal destruction. In a preliminary observation (18), we have already shown the absorption of penicillin from the duodenum of cats to be relatively slow as compared to a satisfactorily absorbed drug such as sulfadiazine. One-tenth millimole of sodium penicillin G per Kg. body weight placed in the ligated duodenum of anesthetized cats was 76 per cent recoverable from that site after one hour of absorption, while sodium sulfadiazine was only 5 per cent recoverable under the same conditions. The present work is a continuation of these preliminary studies.

Since species variation, source of penicillin, and conditions of the experiment would be expected to alter average figures for excretion, we felt it first necessary to establish new averages for urinary excretion from oral and parenteral administrations under our own conditions rather than to use those in the literature from human experiments. Next, a better knowledge of the sites of maximal and minimal penicillin absorption was felt necessary. Lastly, we wished to gain some information on the sites of maximal and minimal destruction in the intestinal tract. Healthy adult mongrel cats deprived of food for twenty-four hours previous to the experiments were used for the study. A single lot of partially purified sodium penicillin with a potency of 1016 units per mg. was used throughout the study.

EXPERIMENTAL

I. Comparison of Oral and Intramuscular Administration

To 15 animals was administered by stomach tube 4500 units penicillin per Kg. dissolved in 2.5 cc. *normal* saline solution per Kg. Absorption was allowed to take place for ten hours. Preliminary experiments showed that urinary excretion as well as serum levels were zero at the end of this period of time, indicating cessation of absorption. All urine voided during the absorption period was collected, and at the end of the period the animal was induced into ether anesthesia. The bladder was removed, thoroughly washed out with 0.1 *M* phosphate buffer, *p*H 7, and the washings pooled with any urine voided during the absorption period. To check for completeness of absorption, blood samples were drawn by sterile heart puncture and also each section of the gastrointestinal tract (stomach, small intestine, and colon) was ligated separately, removed, washed out, and the washings analyzed for penicillin. The urine and gastrointestinal washings were assayed by the cylinder-plate method and the blood serum samples were assayed by the method of Rammelkamp (19). Preliminary experiments on the gastrointestinal contents of twenty-four-hour fasted cats under Dial-urethane anesthesia showed them to contain no substances interfering with the penicillin assay, and that the contents alone gave no ring of inhibition with the test organism.

To 12 animals was administered intramuscularly 4500 units penicillin per Kg. dissolved in 0.25 cc. normal saline solution per Kg. Absorption was allowed to take place for ten hours as with the oral animals, and at the end of the absorption period urinary excretion and blood serum concentrations were determined as before. Results are shown in Table I.

Discussion.—The average urinary excretion of 40% of the administered dose after intramuscular administration is 5.5 times the average of 7.1% excretion after oral administration. This ratio is comparable to figures on comparative excretion following these two routes of administration reported in the literature on human experiments. It is notable that the range in percentage excretion is much greater following oral administration than following intramuscular administration. This indicates greater variation in absorbability from the gastrointestinal tract. The analysis of the gastrointestinal tract after the oral administration showed absorption (and/or destruction) to be practically complete at the end of the ten-hour period. In 12

TABLE I.—URINARY EXCRETION OF PENICILLIN AFTER ORAL AND INTRAMUSCULAR ADMINISTRATION OF 4500 UNITS PER KG. TO CATS

Method of Administration	No. of Cats	Recovery in Urine Average	Recovery in Urine Range	Blood Serum Concentration	Gastrointestinal Recovery
Oral	15	7.1%	1.4–18.2%	Negative—all cases	Traces in colon—3 cases Negative—12 cases
Intramuscular	12	40.0%	29.0–61.0%	Negative—all cases	.....

cases out of the 15, there was no penicillin found in any part of the gastrointestinal tract, while in the remaining 3 cases a small amount was found only in the colon (trace to 0.3% of administered dose).

II. Sites of Maximal and Minimal Gastrointestinal Absorption

Under Dial-urethane anesthesia (0.7 cc. per Kg. Dial with Urethane, Ciba) 4500 units per Kg. sodium penicillin was placed in the ligated esophagus, stomach, duodenum, jejunum, ileum, or colon of cats. The penicillin was previously dissolved in 2.5-cc. normal saline solution per Kg. in order to standardize intestinal fluid volumes and osmotic influences. The bladder was emptied and the urethra ligated just prior to the introduction of the penicillin. The abdominal incision was sutured and absorption allowed to take place for one hour. Body temperature fall was prevented by the application of supplemental heat. At the end of the absorption period, the incision was re-opened, the ligated section removed, and the contents thoroughly washed out for analysis. Blood samples were drawn by sterile heart puncture. The bladder was removed and the contents washed out for analysis. The analysis procedures used were the same as described in Section I. Results are shown in Table II.

Absorption from the stomach was somewhat better than from the esophagus, but did not approach that found in the small intestine. The average urinary recovery of 0.6% of the administered dose is approximately one-fifth the average urinary recovery of 3.3% obtained from the three sections of the small intestine, and the average blood serum concentration of 0.3 u./cc. is approximately one-tenth the average blood serum concentration of 2.9 u./cc. obtained from the small intestine. Maximum absorption as judged by serum concentrations and urinary excretion was found in the three sections of the small intestine with no significant differences among the three. Absorption from the colon was again comparatively low, the average serum concentration of 0.2 u./cc. being approximately one-fifteenth and the average urinary excretion of 0.3% being approximately one-tenth that obtained from the small intestine. However, it is interesting to note that the average recovery of 58% of the administered dose of penicillin from the colon is not much greater than the average recovery of 54% from the three sections of the small intestine. This points to rapid destruction in the colon and is further substantiated by the experiments following, which were designed to determine the site of penicillin destruction.

TABLE II.—URINARY EXCRETION, BLOOD SERUM CONCENTRATION, AND GASTROINTESTINAL RECOVERY OF PENICILLIN ONE HOUR AFTER INTRODUCTION OF 4500 UNITS PER KG. INTO VARIOUS LIGATED SECTIONS OF THE GASTROINTESTINAL TRACT OF ANESTHETIZED CATS

Site of Absorption	No. of Cats	Recovery in Urine Average	Recovery in Urine Range	Blood Serum Concentration Average	Blood Serum Concentration Range	Gastro-Intestinal Recovery Average	Gastro-Intestinal Recovery Range
Esophagus	3	0.1%	0–0.2%	...	2 neg., 1 trace	91%	85–98%
Stomach	3	0.6%	0.3–0.8%	0.3 u./cc.	0.1–0.6 u./cc.	81%	61–92%
Duodenum	8	3.1%	1.0–4.7%	2.8 u./cc.	2.0–4.4 u./cc.	63%	44–82%
Jejunum	5	4.4%	3.4–5.4%	2.8 u./cc.	1.4–4.2 u./cc.	51%	39–62%
Ileum	6	2.5%	1.3–5.0%	3.0 u./cc.	1.2–4.7 u./cc.	48%	31–63%
Colon	3	0.3%	0.1–0.5%	0.2 u./cc.	Trace–0.32 u./cc.	58%	51–65%

Discussion.—The high average recovery of 91% of the administered penicillin from the ligated esophagus after one hour indicates the absorption from this organ to be very poor. The low average urinary excretion of 0.1% of the administered amount and negligible blood serum concentrations (two negative and one trace out of three tested) are confirmatory evidence of this fact. These results are at variance with those obtained by Chu, *et al.* (20) on rats. These authors reported maximum absorption from the ligated esophagus as judged by blood levels assayed by the method of Wolohan and Cutting.

III. Sites of Maximal and Minimal Intestinal Destruction

The same standard dose of penicillin as used before (4500 units in 2.5 cc. normal saline solution per Kg.) was placed in the ligated duodenum, jejunum, ileum, and colon of cats under ether anesthesia. The anesthetic was then discontinued, the incision sutured, and absorption allowed to take place for ten hours. Preliminary experiments showed almost complete disappearance of penicillin from the ligated section and the blood to take place in this period of time. All urine was collected during the absorption period and pooled with the bladder washings col-

lected at the end of the absorption period. As a check, the blood as well as the contents of the intestinal section were analyzed for completeness of penicillin disappearance. Results are shown in Table III.

TABLE III.—URINARY EXCRETION AND INDICATED INTESTINAL DESTRUCTION OF PENICILLIN TEN HOURS AFTER INTRODUCTION OF 4500 UNITS PER KG. INTO LIGATED SECTIONS OF THE INTESTINAL TRACT OF CATS

Site of Absorption	No. of Cats	Recovery in Urine		Blood Serum Concentration		Intestinal Recovery	
		Average	Range	Average	Range	Average	Range
Duodenum	4	38%	30–48%	Neg.	Neg.	0.1%	0–0.2%
Jejunum	4	23%	11–37%	Neg.	Neg.	0.8%	0–3.4%
Ileum	4	22%	12–37%	Neg.	Neg.	0.7%	0–2.0%
Colon	4	7%	3–19%	0.01 u./cc.	0–0.03 u./cc.	4.6%	1.6–8.7%

Discussion.—An examination of Table III reveals practically complete absorption and/or destruction to have occurred from the ligated sections in the ten-hour period of the experiment. Only in the ligated colon was any significant amount recovered and this averaged but 4.6% of the administered quantity. That some absorption was still taking place at a slow rate is evidenced by a low blood serum concentration obtained in 2 cases of the 4 tested. An examination of the urinary recovery following disappearance from the ligated section offers definite evidence of a progressive rate of destruction downward in the intestinal tract. The average of 38% urinary recovery after absorption from the ligated duodenum is remarkably close to the average of 40% urinary recovery obtained with the same lot of penicillin and the same dose administered intramuscularly. Thus, little credence can be given to theories proposed to explain the inefficiency of an oral dose because of its rate of entry into the blood stream or because of excessive destruction due to direct entry into the portal circulation. The averages of 23% and 22% urinary recovery after absorption from the jejunum and ileum, respectively, indicate considerably more destruction in these two sites than in the duodenum. This was also evidenced in the recoveries obtained from these sections after one hour of absorption in Section II, in which case the average recovery of 63% from the duodenum was significantly greater than the average recoveries of 51% and 48% from the jejunum and ileum, respectively. Destruction in the colon is definitely greatest since an average of only 7% of the administered amount was found in the urine after the ten-hour experimental period. Of course it must be taken into consideration here that in the case of the colon the absorption was not quite complete since an average of 4.6% was recovered in the ligated colon. However, assuming complete absorption of this amount and 40% urinary recovery of all that was absorbed, it would still not raise the total urinary recovery to more than approximately 9%, which is still far below the average recovery for the other sections of the intestine.

From the evidence presented, and the premise that 40% of penicillin reaching the circulation under our conditions is excreted in the urine, an approximation of the comparative amount of absorption and destruction taking place in the various parts of the intestinal tract can be made. Destruction of penicillin in the duodenum is insignificant and complete absorption can take place providing the sojourn in this site is sufficient. On the other hand, destruction in the jejunum and ileum is considerable, approximately 45% of a given quantity of penicillin present being destroyed before it can be absorbed. Destruction in the colon is greatest. Approximately 80% of a given quantity present in the colon is destroyed before it can be absorbed.

The above findings that absorption from the ligated duodenum is complete when sufficient time is allowed, and that a rapidly progressive rate of destruction downward occurs in the intestinal tract are evidence that the inefficiency of an oral dose is due primarily to incomplete absorption in the upper intestinal tract and subsequent destruction in the lower regions. Research directed toward improving the efficiency of an oral dose would therefore seem most promising along lines to increase the rate of absorption of penicillin in the upper intestine before it reaches the lower regions of more rapid destruction.

CONCLUSIONS

1. Urinary excretion of penicillin following intramuscular administration in cats is 5.5 fold greater than that following oral administration.

2. A greater variability in excretion occurs following oral administration as compared to intramuscular.

3. Absorption and/or destruction is practically complete ten hours following oral administration.

4. Absorption of penicillin from the esophagus does occur, but is of a very low order of magnitude.

5. Absorption is maximum in the small intestine with no significant differences in rate among the duodenal, jejunal, and ileal sections.

6. Absorption from the stomach is one-fifth to one-tenth as rapid as from the small intestine.

7. Absorption from the colon is one-tenth to one-fifteenth as rapid as from the small intestine.

8. Destruction of penicillin in the duodenum is insignificant.

9. Approximately 45 per cent of a given quantity of penicillin present in the jejunum or ileum is destroyed before it can be absorbed.

10. Approximately 80 per cent of a given quantity of penicillin present in the colon is destroyed before it can be absorbed.

11. The inefficiency of an oral dose is due primarily to incomplete absorption in the upper intestinal tract and subsequent destruction in the lower regions.

REFERENCES

(1) Cutting, W. C., Halpern, R. M., Sultan, E. H., Armstrong, C. D., and Collins, C. L., *J. Am. Med. Assoc.*, **129,** 425(1945).
(2) Finland, M., Meads, M., and Ory, E. M., *ibid.*, **129,** 315(1945).
(3) Bunn, P. A., McDermott, W., Hadley, S. J., and Carter, A. C., *ibid.*, **129,** 320(1945).
(4) Free, A. H., Parkes, R. F., and Biro, B. E., *Science*, **102,** 666(1945).
(5) Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, **2,** 177(1941).
(6) Rammelkamp, C. H., and Helm, J. D., *Proc. Soc. Exptl. Biol. Med.*, **54,** 324(1943).
(7) Libby, R. L., *Science*, **101,** 178(1945).
(8) Perlstein, D., Kluener, R. G., Liebmann, A. J., and Dorrel, J., *ibid.*, **102,** 66(1945).
(9) Charney, J., Alburn, H. E., and Bernhart, F. W., *ibid.*, **101,** 251(1945).
(10) Krantz, J. C., Evans, W. E., and McAlpine, J. G., *ibid.*, **101,** 618(1945).
(11) Seeberg, V. P., and Collen, M. F., *ibid.*, **102,** 225 (1945).
(12) Welch, H., Price, C. W., and Chandler, V. L., *J. Am. Med. Assoc.*, **128,** 845(1945).
(13) Burke, F. G., Ross, S., and Strauss, C., *ibid.*, **128,** 83 (1945).
(14) McDermott, W., Bunn, P. A., Benoit, M., DuBois, R., and Haynes, W., *Science*, **101,** 228(1945).
(15) McDermott, W., Bunn, P. A., Benoit, M., DuBois, R., and Reynolds, M. E., *ibid.*, **103,** 359(1946).
(16) McDermott, W., Bunn, P. A., Benoit, M., DuBois, R., and Reynolds, M. E., *J. Clin. Investigation*, **25,** 19(1946).
(17) Cutting, W. C., Luduena, F. P., Fiese, M., Elliot, H. W., and Field, J., *J. Pharmacol.*, **85,** 36(1945).
(18) Seeberg, V. P., Illg, P. L., and Brown, D. J., *Science*, In press.
(19) Rammelkamp, C. H., *Proc. Soc. Exptl. Biol. Med.*, **51,** 95(1942).
(20) Chu, W. C., Fiese, M. J., Cutting, W. C., and Collen, M. F., *ibid.*, **60,** 159(1945).

Apparatus, for Tablet Disintegration, and for Shaking-Out Extractions*

By SOLOMON GERSHBERG† and FERDINAND D. STOLL‡

A mechanical shaking-out apparatus is described and illustrated. This apparatus has wide application in a pharmaceutical laboratory. Its uses in tablet disintegration time tests, shaking-out extraction procedures, and for stirring melting point baths are described.

THE APPARATUS, herein described, has been in use in chemical inspection laboratories of the U. S. Army Medical Department since 1940 and at present in the inspection laboratory of the Army–Navy Medical Procurement office. It has been found to give satisfactory, reproducible results when used for tablet-disintegration tests and is also employed for agitation of bath during melting-point determinations, and for containers during shaking-out extraction procedures, and during development of spectrographic films.

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Its advantages are: (a) Relative simplicity of construction and operation; component parts are available to all laboratories; apparatus is relatively inexpensive to make and operate. (b) Conditions of operation, such as speed, amplitude of movement, intensity of agitation, composition and temperature of bath, can be standardized, easily controlled and are reproducable. (c) Sample of material being tested is constantly in full view of operator; ease of observation of tablets during disintegration-testing irrespective of the resultant turbidity or color of the liquid used in the bath.

The apparatus, (Fig. 1.), as employed for tablet-disintegration tests, consists of the following parts:

APPARATUS

Basket-rack—The basket-rack (Fig. 2) is assembled from two bakelite disks, about 90 mm. diameter and 6 mm. thick, with six concentrically arranged holes to receive glass tubes each 77 mm. length and

24 mm. outside diameter. The tubes are spaced by the disk perforations and are held in place by #10 mesh brass screen attached by three screws to the under surface of the lower disk. They are further secured by a brass plate, 90 mm. diameter, 1 mm. thick, and with six perforations each 20 mm. diameter, and which coincide with the upper open ends of the six glass tubes. The whole assembly is further secured and made rigid with three bolts, and a central shaft, locking the screen, bakelite disks, and brass plate into position with the glass tubes spaced by the perforations. The central shaft extends about 10 cm. above the brass disk, and its upper end terminates in an "eye" to serve for connections to the string which suspends, and raises and lowers the basket-rack. The over-all dimensions of the basket-rack are about 90 mm. in diameter, 80 mm. height, plus the extending length of the central shaft, the distance between the opposing faces of the upper and lower bakelite disks is approximately 65 mm. The weight of the entire basket-rack, including six glass tubes, is approximately 350 Gm.

Beaker—A low form, standard 1000-cc. Pyrex glass beaker serves to contain the bath and the basket-rack when immersed therein. It is supported over the heating device by a wire gauze with an asbestos center. In operation, this beaker is protected from air currents by a shield (not shown in illustration). Shield has a small but sufficient cut out on one side, facing the operator, to permit observation of tablets under test.

Heater.—A gas micro burner, protected from air drafts by a suitable shield.

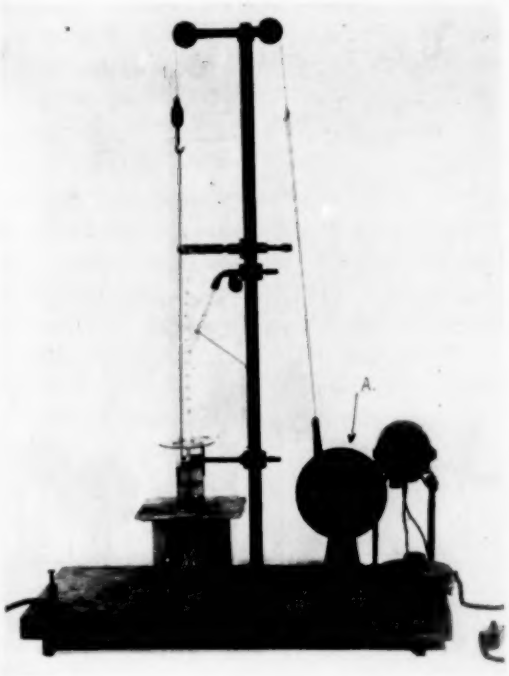

Figure 1

Motor.—An E. & A. Co. $^1/_{80}$-H. P. motor with a built-in, $8^1/_2$-reduction-gear, to reduce the output speed from 5000 r. p. m. to about 600 r. p. m. However, any small motor with a worm-drive and speed-reduction gear to provide satisfactory torque and a controllable output speed of about 20 r. p. m. may be used. A rheostat is employed to aid in controlling the output r. p. m.

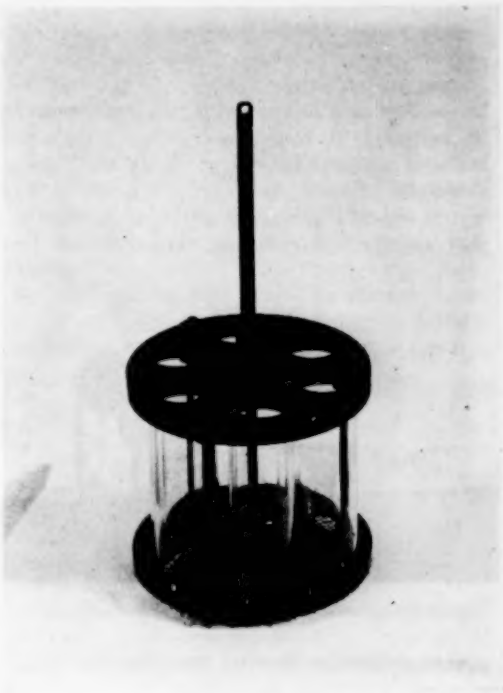

Figure 2

Reduction Gear and Agitator.—(Fig. 1.-A.). This reduces the output speed of the motor shaft to the desired speed of about 20 r. p. m.; by means of a worm gear assembly the rotatory motion of the motor shaft is transmitted to the reduction-gear plate, the axis of which is horizontal and at right angles to the axis of the driving shaft. The reduction-gear plate, about 12.5 cm. diameter, has a projecting pin, excentrically placed on the gear plate. A piece of surgical silk, or fishing line, connects this pin with the "eye" of the central shaft of the basket-rack.

Agitation.—As the reduction-gear plate revolves at approximately 20 r. p. m. the excentric pin on the reduction-gear plate converts the rotatory motion of the plate into an up-and-down movement. This movement is transmitted by the suture or other line over the pulleys to the basket-rack. The position of the excentric pin on the face of the reduction-gear plate is such as to impart a vertical amplitude of approximately 50 mm. to the basket-rack, alternately raising and lowering it in the bath. With the observable sections of the glass tubes being approx-

imately 65 mm., this amplitude is such that tablets resting on the wire bottom of the basket-rack will always remain submerged in the bath as the assembly rises and falls.

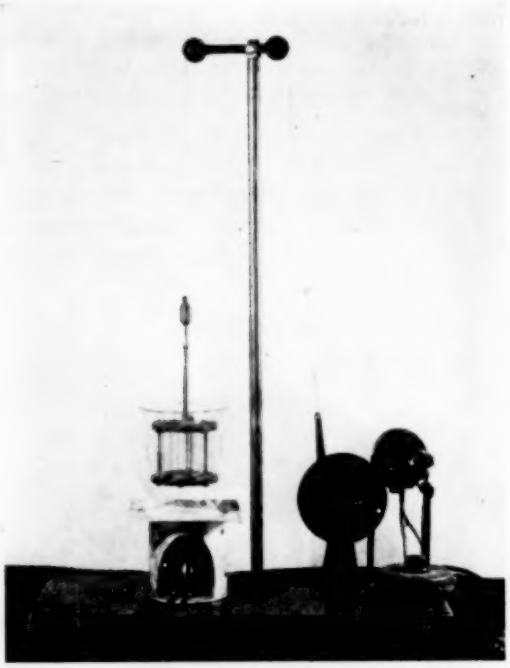

Figure 3

EXPERIMENTAL

Procedure.—About 750 cc. of a liquid (Distilled water, Artificial gastric or Pancreatic fluid, etc.) is warmed to a temperature of about 37°–40°, and placed in the beaker. The basket-rack is then attached to the line from the pulley and its level is adjusted so that when the motor is in operation and the basket-rack rises and falls, the level of the liquid bath remains above the lower bakelite disk of the basket. Thus, any tablets placed in the tubes will always remain submerged in the bath. A shield, not shown in the illustration, is placed around the beaker, heat is applied, and bath temperature and basket motion are adjusted to desired requirements. A test tablet is placed in each of the glass tubes and starting time is recorded. As the tablets in the basket are alternately raised and lowered, the tablets are laved with the test-fluid which, in effect, alternately flows upward then downward around the tablets. Agitation is gentle; no force nor pressure is applied directly to the tablets which are always submerged. The motion of the basket-rack effects constant change of the fluid in contact with the tablets and disintegration particles fall through the screen bottom of the basket toward the bottom of the beaker. As disintegration (or solution) of the tablets proceeds, new surfaces of the tablets are subjected to the action of new portions of the bath liquid. The tablets are observed during the first five minutes of test then at fifteen minute intervals from starting time, and when no more tablet particles remain on the screen, the test is completed.

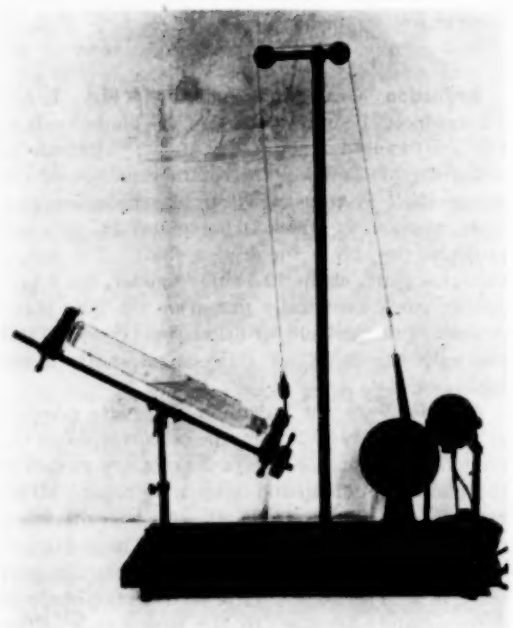

Figure 4a

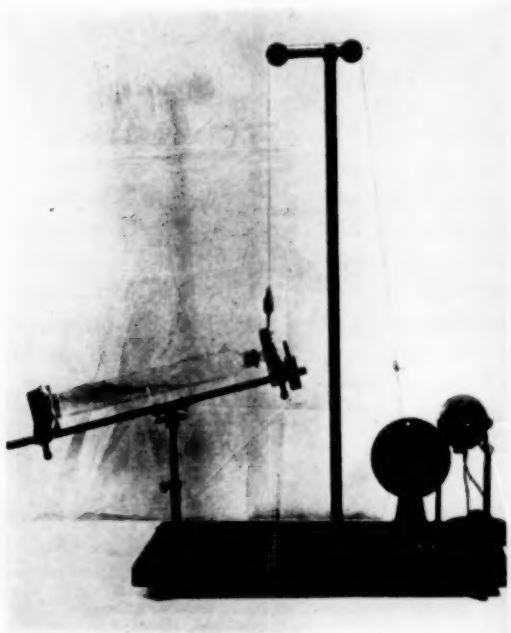

Figure 4b

Stirring Device for Melting Point Determinations.—For this purpose the basket-rack is replaced by a glass rod bent to fit the beaker being used, (see Fig. 3), and this rod is caused to move up and down as was the basket-rack.

Shaking-out Extraction Apparatus.—For this purpose the beaker assembly and basket-rack are replaced by a rocker-arm assembly (Figs. 4a, 4b) one arm of which is connected with the suture from the pulleys. The apparatus is identical to that used for determining disintegration rates for tablets except that a rocker-arm assembly replaces the basket-rack assembly. This rocker-arm assembly consists of:

(a) Telescoping Support, extensibe to .28 cm. from base plate and fitted at the upper end with a metal rocker arm about 15 cm. long. Rocker-arm is securely bolted to a brass rod 1 cm. square, 48 cm. long, about 20 cm. from one end of the rod.

(b) Two wooden plates, approximately 8 cm. square, with a square hole and setscrew for attachment to the square rod. Plates may be adjusted along the rod and fixed by set screws in position to accommodate various sized containers. Inner faces of plates are recessed to accommodate and anchor ends of flasks used.

(c) A metal counterpois, with a setscrew ending in a square hole, is attached to the square rod to balance the assembly when connected to the actuating end of the suture, thus permitting easy rocking of the assembly when all connections are made. Weight and position of counterpois are variable to accommodate variations in weight of apparatus and contents.

Procedure.—A container (cylindrical flask, separatory funnel, etc.) with liquid to be agitated, is centered between the wooden plates on the square rocker-arm and plates are locked into position of optimum balance. Counterpois is attached, if needed. The driving end of the suture is attached to the proximal wooden plate. Rocking amplitude is adjusted by position of the excentric pin on the speed reduction plate and rocking frequency controlled by the rheostat.

The apparatus is also adaptable for use in developing spectrographic plates, permitting uniform agitation and standardized development so essential for quantitative spectrography.

Book Review

Scientific Societies In The United States, by RALPH S. BATES. A Publication of the Technological Press, Massachusetts Institute of Technology. John Wiley & Sons, Inc., New York, 1945. vii + 246 pp. 21.5 x 14 cm. Price, $3.50.

This book belongs to the comparatively small group of publications which, after their appearance, make everybody wonder why they had not been written long ago. The topic is of so much general interest that it would have been gratefully received even if it were nothing more than the title seems to imply: a catalogue of the learned societies and/or the societies of learned people and those interested in the promotion of knowledge and learning.

The fact is that Dr. Bates's book is far from being a mere catalogue. Painting in broad and bold strokes the contemporary scene from the needs and opportunities of which the scientific societies in the United States emerged, Dr. Bates has succeeded in adding sociological to the topical interest. The chapter headings give proof of his special way of approach. An introductory chapter entitled "Scientific Societies in Eighteenth Century America" is followed by chapters bearing the headings: "National Growth, 1800–1865"; "The Triumph of Specialization, 1866–1918"; "American Scientific Societies and World Science, 1919–1944"; "The Increase and Diffusion of Knowledge".

It is understood that in a book of this type, there must be some omissions and unintentional misinterpretations. Dr. Bates has included the AMERICAN PHARMACEUTICAL ASSOCIATION and the State Pharmaceutical Associations side by side with their medical counterparts. The founding of the AMERICAN PHARMACEUTICAL ASSOCIATION is mentioned and motivated as follows: "In 1820 the United States Pharmacopœial Convention held its first meeting, presaging better organization among the pharmacists of the nation and in 1852 the

AMERICAN PHARMACEUTICAL ASSOCIATION was formed" (p. 67)?* There was no "United States Pharmacopœial Convention" until 1900. There was not one pharmacist among the delegates who formed the "General Convention" of January 1, 1820. For the relationship between the Pharmacopœia of the United States and "better organization among the pharmacists of the nation," the founding of the first local American pharmaceutical association, the Philadelphia College of Pharmacy, only a few months after the issuance of the first U. S. P. would have been a much better proof than the establishment of the AMERICAN PHARMACEUTICAL ASSOCIATION in 1852.

The author lists the AMERICAN PHARMACEUTICAL ASSOCIATION together with the American Medical Association, the American Chemical Society, and a number of "similar" groups among what he calls the "horizontal combination" of scientific societies representing "a federation of regional, state, and local societies devoted to one particular branch of science" (p. 177). As far as the AMERICAN PHARMACEUTICAL ASSOCIATION is concerned, that is not quite correct. The A. PH. A. was founded by representatives of "regional societies," has promoted the establishment of such groups and has tried to bind them organizationally to the older organization. It was, however, in steadily growing measure individual membership on which the national association was based primarily, and since September 1, 1920, even the article (V) of the By-Laws of the A. PH. A. entitling "all local and state organizations of Pharmacists to delegates as their representatives in the annual meeting" has been deleted. The idea not only of "a federation of regional, state, and local societies," but of special national associations of the drug trade also, has found its materialization since 1912 not in a transformation of the A. PH. A. into a roof organization, but in a special institution within the A. PH. A., the House of Delegates, one of the most brilliant brain childs of a most brilliant man, Dr. James H. Beal. In this institution, too, personal membership in the A. PH. A. in addition to delegation from one of the associations concerned carries the right to vote.

On page 118 the following statement has been made: "Pharmacologists too were meanwhile organizing numerous state and local professional societies [a footnote lists a number of State Pharmaceutical Associations founded between 1874 and 1899, the dates not always being correct]. A national wholesale druggists' association was founded in 1876. The National Association of Boards of Pharmacy was organized in 1904. The American Society for Pharmacology and Experimental Therapeutics was organized in 1908."

Here the confusion between pharmacy and pharmacology, so frequent in literature, becomes once again obvious. It was pharmacists who founded the pharmaceutical associations, and it was pharmacologists, i.e., people testing and checking the action of drugs, who organized the last-mentioned society.

With no explanation given for the mentioning of the National Wholesale Druggists' Association and the National Association of Boards of Pharmacy in a book devoted to scientific societies, the conclusion seems justified that it was the educational endeavor in which, under the leadership of Dr. E. L. Newcomb, the first has excelled and to which the second is committed, that caused this distinction. All the more, however, the American Association of Colleges of Pharmacy seems entitled to the same honor. The National Conference on Pharmaceutical Research and the American Institute of (not "for") the History of Pharmacy are mentioned (p. 151). The American Drug Manufacturers' Association is listed as a "technical" society (p. 183). Among the many botanical and plant science societies mentioned, the Plant Science Seminar is missing.

The work done by Dr. Bates in not only collecting but connecting the enormous amount of data presented by him is admirable. The few comments made by this reviewer are supplementary rather than critical. It proves Dr. Bates's knowledge and objectivity that in his book pharmacy has been given full recognition as to its part in the organized endeavor in the building up of society.—GEORGE URDANG.